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EPIDEMIOLOGICAL OBSERVATIONS ON LEPTOSPIROSIS IN NORTH QUEENSLAND[1]

E. H. DERRICK, D. GORDON,[2] C. J. ROSS, R. L. DOHERTY, C. N. SINNAMON, V. M. MACDONALD AND J. M. KENNEDY[3]

From the Queensland Institute of Medical Research, Brisbane

THIS paper analyses the circumstances attending the infection of 152 persons with leptospirosis in North Queensland (Figure I). The cases were diagnosed during the first two years' work of the Innisfail Field Station of the Queensland Institute of Medical Research—that is, from July, 1951, to June, 1953. They will be discussed from the following points of view: (i) the method of selection; (ii) geographical distribution; (iii) seasonal incidence; (iv) association with rainfall; (v) age and sex; (vi) occupation.

THE SELECTION OF CASES

By special arrangement, cases of fever were notified by medical practitioners to the staff of the Field Station, who then investigated them.

The diagnosis of leptospirosis was established either by culture of leptospiræ from the blood of the patient or by demonstrating a significant rise in the agglutination titre of paired sera. (One case was accepted on a significant fall in titre.) The finding of leptospiral agglutinins in a single specimen of convalescent serum was not accepted as proof of the leptospiral nature of the current illness, as these might have resulted from a previous infection.

Cultures were inoculated and incubated at Innisfail. When leptospiræ were isolated, the cultures were transferred to the Laboratory of Microbiology and Pathology, Brisbane, for provisional identification, and thence to Dr. J. C. Broom, Wellcome Laboratories of Tropical Medicine, London, for final typing. Agglutination tests on patients' sera were also carried out in the Laboratory of Microbiology and Pathology, and we would express our considerable indebtedness to the Director, Dr. J. I. Tonge, and his staff for their indispensable cooperation. Experiences with cultural and agglutination methods, including the isolation of leptospiral types new to Queensland, are being reported separately (Sinnamon *et alii*, 1953; Smith *et alii*, 1954; others to follow).

TABLE I

Types of Leptospiral Infection in North Queensland, 1951–1953

Infecting Serotype of Leptospira	Cases Diagnosed by Culture	Cases Diagnosed by Agglutination	Total
Icterohæmorrhagiæ ..	2	2	4
Canicola	6	2	8
Australis A	15	15	30
Australis B	22	13	35
"Robinson"	6	6	12
Pomona	2	0	2
Hyos[1]	8	5	13
Medanensis	2	0	2
"Kremastos"	14	6	20
"Szwajizak"	4	3	7
"Celledoni"	4	7	11
Type not determined ..	—	8	8
Total	85	67	152

[1] This leptospira has been commonly known as *L. mitis* Johnson. The International Committee on Bacteriological Nomenclature has decided, because of a prior use of *mitis*, to accept instead the name *L. hyos* Savino and Rennella.

The leptospiral strains isolated have been provisionally classified in eleven serotypes as shown in Table I. When the diagnosis was made by agglutination, identification of the infecting type was often difficult because of

[1] Received on January 11, 1954.

[2] Director of Industrial Medicine, Queensland Health Department, Brisbane.

[3] Inspector in Charge, Weil's Disease Control.

confusing cross-agglutination, and the classification was to some extent tentative. In some instances, even a tentative identification of type was impracticable.

These 152 cases are not a complete record. There were some probable leptospiral infections about which information was inadequate, and we have reason to believe there were other cases, particularly among the mild, uninvestigated attacks of fever in subjects not covered by compensation.

None of these patients died; but it may be mentioned that a diagnosis of leptospirosis was strongly suspected, although not confirmed in the laboratory, in the case of a canecutter who died in August, 1952.

GEOGRAPHICAL DISTRIBUTION

The Innisfail Field Station draws its material from a coastal strip of North Queensland extending from Ingham (18° 40′ south) to Cooktown (15° 30′ south), a distance of 230 miles (Figure I). Topographically the area presents a series of mountain ranges and tablelands separated from the Pacific Ocean by a narrow coastal plain. The highest peak is Mount Bartle Frere, 5275 feet. Many rivers and creeks rise in the mountains and intersect the plain (Figures II and IV). An area of intense rainfall includes Tully (where the annual average is 176 inches), Innisfail and Babinda. To north, south and west of this area, the rainfall sharply declines. Representative rainfalls are noted in Table II. The flora varies, according to soil, rainfall and elevation, from dense tropical jungle to light hardwood forest.

For our purpose this area may be classified in four regions: (i) the tablelands; (ii) the city of Cairns; (iii) the coastal plain, apart from Cairns; (iv) the Cooktown district.

The Tablelands

The tableland shires of Herberton, Atherton, Eacham and Mareeba, at an elevation of 1500 to 3000 feet, support a population of 19,500, which is occupied with the farming of many kinds of crops, dairying, timber-getting and mining. They have supplied only two cases of leptospirosis in our series. One patient was a dairy farm worker of Julatten, who was infected with *Leptospira australis B*; the other was a wolfram miner of Mount Perseverance near Julatten, whose infection was caused by *L. hyos*. Another wolfram miner from Mount Perseverance, also infected with *L. hyos*, had his claim on the other side of the range, which lies in Douglas Shire. (We have also reports of at least four other cases of leptospirosis, not

FIGURE I

Map of the high rainfall area of North Queensland. Isohyets are represented by broken lines. Shires are indicated as follows: A, Atherton; C, Cook; CL, Cardwell; D, Douglas; E, Eacham; H, Herberton; HK, Hinchinbrook; J, Johnstone; M, Mareeba; 1, 2, 3, 4, Divisions of Mulgrave.

included in our series, among residents of Eacham and Atherton shires during the year 1952–53. The types concerned were *pomona* (two cases), *hyos* and *australis B.*)

FIGURE II

A characteristic landscape in the area where leptospirosis is endemic—mountain (Mount Bartle Frere), river (Russell River) and canefield.

The City of Cairns

The city of Cairns has a population of 19,000. None of our cases of leptospirosis are known to have arisen within its boundaries, though several of its citizens have become infected close by in Mulgrave shire.

The Coastal Plain

The important region for leptospirosis is the narrow coastal plain. This includes, as well as the city of Cairns, the shires of Hinchinbrook, Cardwell, Johnstone, Mulgrave and Douglas. The livelihood of the 42,000 people of these shires depends almost entirely on sugar production.

The distribution of cases according to the shire in which the infection originated is set out in Table II. Although Hinchinbrook shire, centred at Ingham, was the site of an outbreak of leptospirosis in 1933–1934—the first to be recognized in Australia—this shire has not contributed any cases to our series. Five only came from Cardwell shire.

The adjoining shires of Johnstone and Mulgrave contributed three-quarters of the cases. While this preponderance may have been influenced by proximity to the field station, it certainly indicates a high endemicity there. Within the shires the distribution was by no means even. In Mulgrave, a relative concentration of cases was found in Division 3, which includes the township of Babinda and the surrounding districts. Here the rate per thousand of population was 16·7, compared with 5·8 for the whole shire and 3·7 for Johnstone. The Johnstone rate is no doubt reduced by the inclusion within the shire of an urban population of 7000 at Innisfail.

TABLE II

Geographical Distribution of Leptospirosis in North Queensland, 1951–1953

Area	Estimated Population (June 30, 1952)	Number of Cases	Cases per 1000 Population	Average Annual Rainfall (Inches) and Recording Station
Tableland shires:				
Herberton	3400	0		44, Herberton
Atherton	4750	0		55, Atherton
Eacham	4000	0		67, Malanda
Mareeba:				
Division 4	1100 } 7330	2 } 2	1·8	60, Julatten
Rest of shire	6230 }	0 }		35, Mareeba
Cairns City	19,000	0		88, Cairns
Coastal shires:				
Hinchinbrook	10,000	0		79, Ingham
Cardwell	5000	5	1·0	176, Tully
Johnstone	13,631	51	3·7	142, Innisfail
Mulgrave:				
Division 1	2060 } 11,220	5 } 65[1]	2·4 } 5·8	80, Hambledon
Division 2	4510 }	13 }	2·9 }	76, Grodonvale
Division 3	2750 }	46 }	16·7 }	163, Babinda
Division 4	1900 }	0 }	0 }	
Douglas:				
Daintree	150 } 2600	4 } 15	26·7 } 5·8	107, Daintree
Miallo *et cetera*	300 }	6 }	20·0 }	96, Miallo
Rest of shire	2150 }	5 }	2·3 }	91, Mossman
Cook shire	1250	7	5·6	68, Cooktown
Unallotted to shire[2]	—	7	—	—

[1] Includes one case not allotted to a Division.

[2] In these cases it was not clear from the history in which shire the infection originated.

The shire of Johnstone has no administrative divisions, but it includes the areas supplying three sugar mills, and these may be used as a basis of comparison as far as 35 canefield workers are concerned. These were apportioned as follows: Goondi area seven, South Johnstone area 18, Mourilyan area 10. Thus all three areas were represented. In the series of Kennedy *et alii* (1952) there were no cases from Mourilyan area, but 34 from Goondi and 13 from South Johnstone. Of the present ten

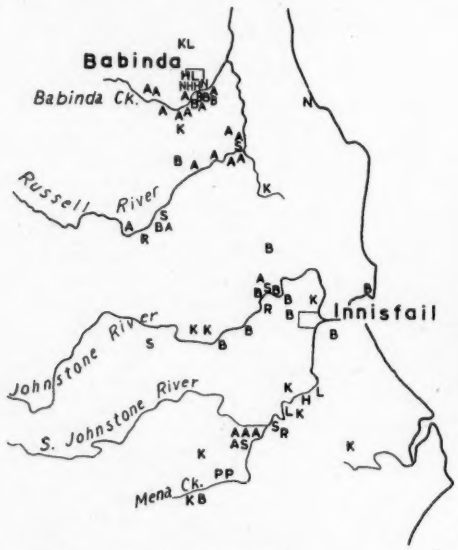

FIGURE III

Part of Mulgrave and Johnstone shires, showing the probable sites of origin of 70 cases of leptospirosis. There is a strong preference for the vicinity of rivers and creeks. "A" indicates site of origin of a case of *australis A* infection; "B", *australis B*; "H", *hyos*; "K", "Kremastos"; "L", "Celledoni"; "N", *canicola*; "P", *pomona*; "R", "Robinson"; "S", "Szwajizak".

Mourilyan cases, eight were caused by "Robinson", "Kremastos" and "Celledoni" serotypes, and infections by these new types would not have been recognized at the time of the earlier series.

Figure III shows the probable sites of infection in 70 cases. The proximity of most of these to rivers and larger creeks is noticeable. In assessing the significance of this distribution, it needs to be kept in mind that it is the flatter country which has been selected for sugar farms, so that much of the settlement is along river valleys. However, many farms are comparatively elevated and well drained, and it is rare for infections to occur on them. On the other hand, certain low-lying river flats have earned a reputation for leptospirosis. For instance, at the junction of Mena Creek with the South Johnstone River is a corner of low-lying ground, liable to flooding in wet weather (Figure IV). The two farms which occupy this area have supplied five cases to our series, as well as others from time to time in previous years.

Douglas shire lies to the north of, and is widely separated from, the other sugar-growing areas. Its principal town is Mossman and its fevers have been described as "Mossman fever" (Clarke, 1913). It seems likely that "Mossman

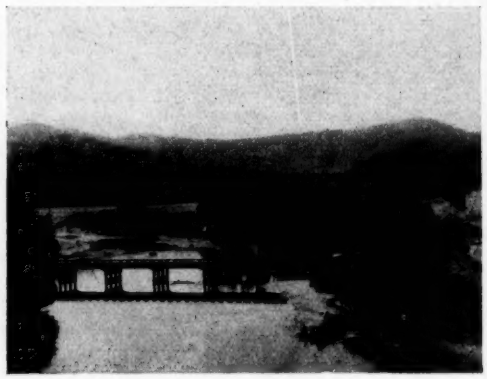

FIGURE IV

Canefields beside the South Johnstone River where five cases of leptospirosis originated.

fever" is a composite term and includes leptospirosis and scrub typhus as important constituents. Leptospirosis has comprised about a third of the cases of fever that we have studied from the Mossman area. As is noted in Table II, the 15 cases in the present series were not evenly distributed throughout the shire. The incidence at Daintree, where the small community engages in dairying and cattle grazing, and in the Miallo district—a cane-growing area—was significantly higher than in the rest of the shire.

The Cooktown District

The fourth region consists of lightly inhabited, rugged country extending for about 40 miles to the south of Cooktown, being part of Cook shire. Here are some scattered tin mines and saw mills. Our seven cases of leptospirosis—the first to be reported from this area—present some points of occupational interest which are discussed later.

TABLE III

Distribution of Leptospiral Types by Shires[1]

Type of Leptospira	Mareeba	Cardwell	Johnstone	Mulgrave, Divisions 1, 2, 4	Mulgrave, Division 3	Douglas	Cook	Unallotted to Shire	Totals
Icterohæmorrhagiæ	—	—	—	3 (2)	1	—	—	—	4 (2)
Canicola	—	—	1	2 (2)	3 (2)	1 (1)	1 (1)	—	8 (6)
Australis A	—	1	5 (4)	3 (1)	19 (10)	1	—	1	30 (15)
Australis B	1	1	13 (11)	4 (4)	9 (6)	2	3 (1)	2	35 (22)
"Robinson"	—	2	8 (5)	—	1 (1)	—	—	1[2]	12 (6)
Pomona	—	—	2 (2)	—	—	—	—	—	2 (2)
Hyos	1	—	1 (1)	3 (3)	5 (3)	3 (1)	—	—	13 (8)
Medanensis	—	—	—	—	—	2 (2)	—	—	2 (2)
"Kremastos"	—	—	13 (9)	1 (1)	3 (2)	1	—	2 (2)	20 (14)
"Szwajizak"	—	—	4 (3)	—	2 (1)	1	—	—	7 (4)
"Celledoni"	—	—	3 (3)	—	3	1	2	2 (1)	11 (4)
Type undetermined	—	1	1	2	—	3	1	—	8
Totals	2	5	51 (38)	18 (13)	46 (25)	15 (4)	7 (2)	8 (3)	152 (85)

[1] The figures in parentheses represent the number of cases in which leptospiræ were cultured.
[2] Mulgrave shire, unallotted to division.

Geographical Distribution of Leptospiral Serotypes

An analysis of leptospiral type in relation to locality is set out in Table III. It shows that infections with each type, except the least frequent, are widely distributed.

The commonest type, *australis B*, made a regular contribution to the infections in every shire in which we found leptospirosis. It ranged from Tully River in Cardwell shire to Rossville in Cook shire, and from coastal plain to tableland.

Australis A, though extending from Tully to Mossman, was relatively concentrated around Babinda. It provided 20% of the total cases and 41% of those in Division 3 of Mulgrave shire.

The types "Kremastos" and "Robinson" were relatively frequent in Johnstone shire.

In each locality where leptospirosis was found a multiplicity of types was the rule, and often the profusion of types was notable. Thus eight types were represented among the 16 infections that originated near the South Johnstone River and its tributary Mena Creek, and six types were responsible for 19 infections close to Babinda. On the other hand, six of the seven cases that occurred along a three-mile stretch of the Russell River were caused by *australis A* (Figure III).

Confirming the local profusion of types, three of our patients suffered during the two-year period under review from two successive infections with different leptospiral types—"Celledoni" and "Kremastos", *australis B* and *hyos*, *australis B* and *australis A* respectively. The serum of many other patients showed evidence of previous infection with heterologous leptospiral types.

Animal studies, which are yet at an early stage, have given serological evidence of *australis A* in dogs in Mulgrave, Douglas (at Daintree) and Cook shires and in bandicoots (*Isoodon* or *Perameles*) at Daintree; of "Szwajizak" in a bandicoot at Daintree; of "Celledoni" in a bandicoot in Herberton shire; and of *medanensis* in a possum (*Trichosurus*) from either Atherton or Herberton shire.

To place in perspective our records of type distribution in North Queensland, it may be noted that *icterohæmorrhagiæ*, *canicola*, *pomona* and *hyos* are well known to be world-wide. The same may also be claimed for *australis A*. Since its original discovery in Ingham, North Queensland (Lumley, 1937), it has been found to be a cause of human infection in Switzerland and Germany (Gsell, 1952; Wiesmann, 1952) and in Indo-China (Kolochine-Erber *et alii*, 1952); it has been isolated from dog and rat in Indonesia and serologically implicated in one human case there (Collier, 1948); agglutinins for it have been found in bovine sera from Silesia (Kathe, 1943), from Japan (Yamamoto, 1951) and from New South Wales (Tonge, 1953). The original isolation of *medanensis* was from a dog in Sumatra (Collier, 1948). *Australis B* is a cause of rice field leptospirosis in Italy (Babudieri, 1953).

SEASONAL INCIDENCE

The seasonal incidence is shown in Table IV. At least one case occurred in every month of the two-year period. When the incidence was analysed by quarters, the highest was found in the January-March quarters. In 1952 this peak was a minor one; in 1953 it was striking. It is in the January-March quarter that rain falls most abundantly in coastal North Queens-

TABLE IV

Monthly and Quarterly Incidence of Leptospirosis in North Queensland

Quarter	Number of Cases 1951	1952	1953	Average Rainfall at Innisfail for 57 Years (Inches)	Average Temperature at Innisfail for 23 Years (Degrees Fahrenheit)
January	—	8	20	20·04	80·0
February	—	6	17	22·65	79·4
March	—	8	12	26·73	78·1
Total first quarter	—	— 22	— 49	— 69·42	— 79·2
April	—	6	13	19·95	75·2
May	—	5	1	12·42	71·2
June	—	1	2	7·23	68·1
Total second quarter	—	— 12	— 16	— 39·60	— 71·5
July	1	6	—	4·75	66·6
August	1	5	—	4·91	67·6
September	6	4	—	3·52	70·6
Total third quarter	— 8	— 15	—	— 13·18	— 68·3
October	6	4	—	3·22	73·8
November	4	6	—	6·37	76·8
December	4	6	—	11·70	79·0
Total fourth quarter	— 14	— 16	—	— 21·29	— 76·5
Totals	22	65	65	143·49[1]	—

[1] The annual average of 142 inches, as given in Table II, is a more recent figure.

land, and this quarter is also the warmest, except that December is warmer than March. For comparison, average rainfalls and temperatures at Innisfail are included in Table IV.

The highest incidence of leptospirosis in North Queensland has not always been in the summer. The original Ingham outbreak had its maximum in July and August—a fact associated by Cotter and Sawers (1934) with the opening of the canecutting season. Kennedy *et alii* (1952) record an explosive outbreak in June, 1950.

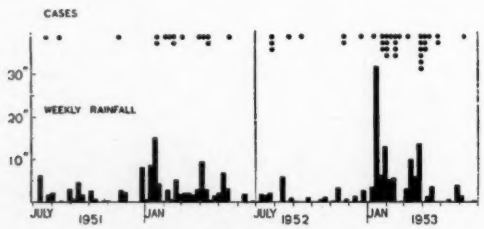

FIGURE V

Histogram showing the time relation of cases of leptospirosis in Johnstone shire to the rainfall at Innisfail. It demonstrates the frequent occurrence of cases soon after heavy rain.

ASSOCIATION WITH RAINFALL

All the localities in which infection occurred have an average annual rainfall of at least 60 inches, most of them much more. At Innisfail and Babinda, the surrounding districts of which supplied most of the cases, the rainfalls are 142 and 163 inches. Daintree, with 107 inches, is also well-watered. The present figures do not, however, establish a linear relation between the incidence of infection in an area and its average annual rainfall. Cardwell shire, for instance, provided few cases in spite of a rainfall of 176 inches at Tully, its centre of population.

The occurrence of cases of leptospirosis shortly after heavy rain—an observation made in 1934 by Morrissey at Ingham—has been repeatedly noted by us. The upsurge of cases in

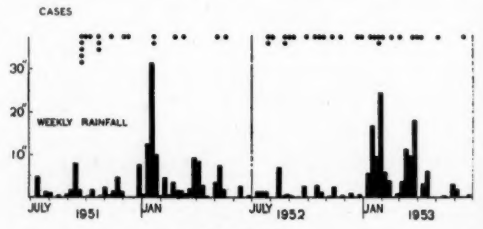

FIGURE VI

Histogram showing the time relation of cases of leptospirosis in Division 3 of Mulgrave shire to the rainfall at Babinda. There are some examples of the occurrence of cases after rain, but they are not as common as in Figure V.

January, 1953, followed the abrupt onset of a heavy wet season. Innisfail recorded its first rain for the year on January 10, 16 inches on January 13, and 51 inches for the month. There were widespread floods throughout coastal

North Queensland. Nine cases of leptospirosis had their onset in the last week of January and six in the first week in February. A lull in cases followed a dry period in the second half of February, and a fresh outbreak followed rains in March.

An association with rain was clearly noticeable on some occasions when isolated wet spells occurred during the drier months. Thus the six wet days, September 12 to 17, 1951, during which eight inches were recorded at Babinda, were followed by six cases of leptospirosis between September 23 and 28. Another rainy period, August 15 to 19, 1952 (seven inches at Babinda), was followed by four cases from August 25 to 29. Other examples may be observed in Figure V, which shows the weekly rainfall at Innisfail together with the weekly incidence of cases in Johnstone shire, and in Figure VI, which gives the rainfall at Babinda in relation to cases in Division 3 of Mulgrave shire.

The association between weekly rainfall and weekly incidence of cases was examined by means of the correlation coefficient (Figure VII). The best correlation in Johnstone shire was found when the weeks were so chosen that the incidence of cases was compared with the rainfall twelve[1] days earlier—that is to say, when the number of cases arising in the week ending July 21 was compared with the rainfall in the week ending July 9, and so on. On either side of the maximum, the correlation was also highly significant at all intervals from eight to eighteen days.

During the calculation of the coefficients, it was noticed that the figures were dominated by the obvious association of cases with the heavy rains in the period January to March, 1953. If this period is excluded by choosing for analysis the eighteen months from July, 1951, to December, 1952, there is still a highly significant correlation, though the maximum coefficient is less and the best correlation is with the rainfall six to twelve days earlier.

In Mulgrave 3, the correlation between cases and rainfall was much less striking, but it was significant when the intervals were ten and eleven days. It may be noted that Mulgrave 3, unlike Johnstone, did not show an excess of cases in the wet seasons, either of 1952 or of 1953.

If the incubation period for leptospirosis in North Queensland is similar to that which Schüffner (1934) observed for infection with *L. icterohæmorrhagiæ* in Holland (four to 19 days, with a mean of $10{\cdot}4 \pm 3{\cdot}1$), there must, after a heavy fall of rain, be a rapid development of conditions favourable to human infection. It is not, of course, falling rain in which leptospiræ will occur, but water on the ground in pool or watercourse. How long rainwater persists in the environment after falling will depend on such factors as rapidity of run-off, degree of saturation of the soil and porosity of soil.

The association with rainfall was further studied by noting the rainfall during the possible period of infection for each case that arose in

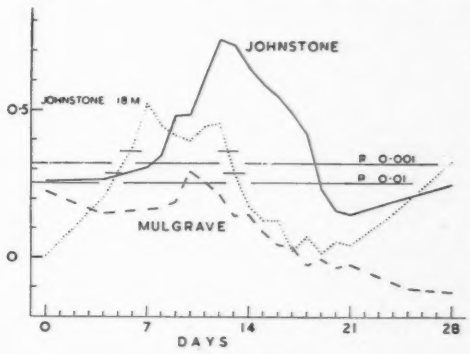

FIGURE VII

Correlation between the incidence of leptospirosis and the rainfall from nil to twenty-eight days previously. For comparison with the weekly rainfall, the cases were grouped in moving seven-day periods. A striking correlation is shown in Johnstone shire, with rain eight to eighteen days previously, particularly at twelve days. A significant, but less striking, correlation is shown in Division 3 of Mulgrave shire at ten days. (There might be some doubt as to whether the levels given for probability are applicable in view of the nature of the variables correlated.)

Johnstone shire or Mulgrave 3. The rainfall figure used for an individual case was the highest that could be obtained by selecting any seven consecutive days during the period four to nineteen days before the onset. As before, the rainfall at Innisfail was used for Johnstone cases and that at Babinda for Mulgrave 3. The combined mean weekly rainfall for the two places for the two years was 2·65 inches.

In 80% of cases the rainfall as defined exceeded 2·65 inches, and in nearly half the cases it exceeded thrice this figure (Table V). However, there were 19 cases (20%) in which the rainfall was unusually low, and these deserve study. With some there was practically no rain throughout the whole period of four to

[1] More accurately 12½ days, as the rainfall recorded for a particular day is the amount of rain that fell during the twenty-four hours ending at 9 a.m. on that day.

nineteen days. The 19 cases did not show any apparent relation to the age or occupation of the patient or to the infecting type of leptospira, five types — *australis A*, *australis B*, " Kremastos ", *hyos* and *canicola*—being represented.

However, cases associated with very heavy rain were relatively more common in Johnstone shire, and those associated with dry weather relatively more common in Mulgrave 3 (χ^2 for Table V = 8·28, $P < 0{\cdot}02$). The explanation of this difference awaits further study. It is not apparently due to the presence among the Johnstone cases of more " Kremastos " and " Robinson " and fewer *australis A* infections than among those from Mulgrave 3. As regards age and occupation, the cases from the two shires were fairly comparable.

TABLE V

Rainfall at the Likely Period of Infection

Area of Origin of Cases	Greater than 7·95 Inches	2·65 to 7·95 Inches	Less than 2·65 Inches	Total
Cases in Johnstone shire	30	16	5	51
Cases in Division 3 of Mulgrave shire	16	16	14	46
Total ..	46 (47%)	32 (33%)	19 (20%)	97

In at least six of the dry weather cases the patients had not avoided association with water. Four had a history of spear-fishing or swimming. One man had been clearing drains around his cane farm. A salesman had cleaned out a sump and drain at his residence.

AGE AND SEX

The ages of the patients (Table VI) ranged widely, from six years (a boy with a *canicola* infection) to sixty-eight years (a retired farmer infected with " Celledoni "). The highest incidence was in the third decade of life, and 89% of the patients were men in the working years (fifteen to fifty-nine). This suggests a strong occupational leaning. There were 14 children aged under fifteen years, and six females (including two children). The age incidence of infection with the individual leptospiral types also ranged widely. The average age of the *australis A* patients was slightly but significantly greater than that of *australis B* or " Kremastos " patients.

LEPTOSPIROSIS AND OCCUPATION

By far the largest occupational group in the series (Table VII) was that associated with the canefields. Smaller but noteworthy groups were schoolboys, timber getters and scrub clearers, bridge and tramway workers and miners.

Canefield Leptospirosis

Canefarmers, canecutters and canefarm labourers comprised 87 patients or 57% of the total. To these might have been added a

TABLE VI

Leptospirosis : Age and Sex Distribution

Age (Years)	Male Subjects	Female Subjects	Total	Type of Leptospira					
				Australis A	*Australis B*	" Robinson "	*Hyos*	" Kremastos "	" Celledoni "
5 to 9	5	1	6	—	2 (1F)	—	2	1	—
10 to 14	7	1	8	—	2 (1F)	2	—	1	1
15 to 19	17	1	18	4	3	—	—	7 (1F)	1
20 to 24	26	—	26	5	11	3	—	3	1
25 to 29	30	—	30	3	10	3	4	2	—
30 to 34	16	—	16	5	2	1	1	2	3
35 to 39	12	—	12	5	1	1	—	1	2
40 to 44	20	1	21	6	3	—	3 (1F)	1	1
45 to 49	5	1	6	1	—	1	—	1	—
50 to 54	2	1	3	1	—	1	1 (F)	—	—
55 to 59	3	—	3	—	1	—	1	—	1
60 to 64	—	—	—	—	—	—	—	—	—
65 to 69	3	—	3	—	—	—	1	1	1
Total ..	146	6	152	30	35	12	13	20	11
Mean age[1] (years) ..	—	—	29·4	31·83	25·29	28·25	35·08	24·75	35·18
Standard deviation ..	—	—	12·53	9·50	9·92	11·75	17·03	13·35	15·40

[1] The difference between the mean ages for *australis A* and *australis B* is 2·7 times the standard error. The difference between the mean ages for *australis A* and " Kremastos " is 2·1 times the standard error.

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There are about 4000 canecutters employed in the region under review. Some of these are employed on the canefarms between harvests; the majority are not, and the entry of this additional labour force into the canefields about May each year is an important factor in the incidence of leptospirosis. Cutting continues until about December—the exact dates of its beginning and ending vary from year to year and from mill to mill.

TABLE VII

Leptospirosis: Occupation in 152 Cases

Type of Occupation, or Group	Number of Cases
Associated with canefields:	
Canefarmers (34), canecutters (29), canefarm labourers (24)	87
Associated with farms, other than canefarms:	
Dairy (4), grazing (1), retired and tending a few bananas (1)	6
Timber getters (7), scrub clearers (4)	11
Bridge and tramway workers	7
Sugarmill employees	5
Miners	5
Various:	
Unemployed (2), sawmill employee, teacher, butcher, saltwater fisherman, motor mechanic, herdsman, salesman, laundryman, breadcarter	11
Schoolboys (14), schoolgirls (2)	16
Females (other than schoolgirls):	
Housewives (3), dental attendant (1)	4
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There were rather more canefarmers infected than canecutters, and rather fewer canefarm labourers. It may be noted that cutting is done in the drier months, while other activities on the farm continue in the wet season, and that specific control measures are more applicable to canecutting than to other farm work. Canefarmers and farm labourers, questioned about their activities before their illness, often stated simply that they had been engaged in general farm work. Sometimes they specified clearing scrub, grass or weeds, cleaning out drains or cutting cane for planting. The most common feature in their histories, as with the cutters, was previous wet weather.

The incidence of leptospirosis as reported here may have been modified in some degree (we would like to think considerably) by the efforts of the Weil's Disease Campaign to control the disease. Practically all cane is burnt before being cut (Figure VIII). This is done, as a rule, for other than health reasons; but it has the useful effects of destroying exposed leptospiræ, evaporating surface moisture and destroying or driving away rats. Inspectors may forbid the cutting of rat-infested cane which by reason of wet conditions has not been effectively burnt. The use of protective footwear is encouraged, so that lacerations on the feet will not facilitate the entry of leptospiræ.

An association of leptospirosis with canefields in North Queensland has been apparent since 1933. The earliest cases to be recognized were all in canefield workers, and for a time the disease in North Queensland was thought to be restricted to them. However, about 40% of our cases were not associated with cane, and the independence from canefields is emphasized particularly by the cases among miners at Cooktown, dairy farmers at Daintree and bridge workers on the upper reaches of the Tully River. All these places are many miles from a canefield.

FIGURE VIII

Cutting sugar cane. The cane is burnt prior to being cut—that is, the dead leaves burn; the stalk remains undamaged, and its content of sugar is not appreciably diminished provided that it is milled within about forty-eight hours. One at least of the gang is barefooted.

Timber Getters and Scrub Clearers

In April, 1953, a party of eight men was clearing scrub in swampy country about five miles south of Cairns, preparing for the erection of an electric power line. Three became infected, two with *icterohæmorrhagiæ*, one with *australis B*. The other scrub clearer had been clearing the way for a road in Cook shire.

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The seven timber getters had been working in at least six localities. Their histories did not indicate their mode of infection.

Bridge and Tramway Workers

In March, 1952, three members of a gang became ill within three days of one another after working in water on the construction of a road bridge over the Tully River (Figure IX). Two had " Robinson " infections ; with the third the agglutination test did not distinguish between " Robinson " and *australis B*. A month later another bridge worker from the same camp was infected with *australis B*. The " Kremastos " type infected a bridge foreman working near Mossman.

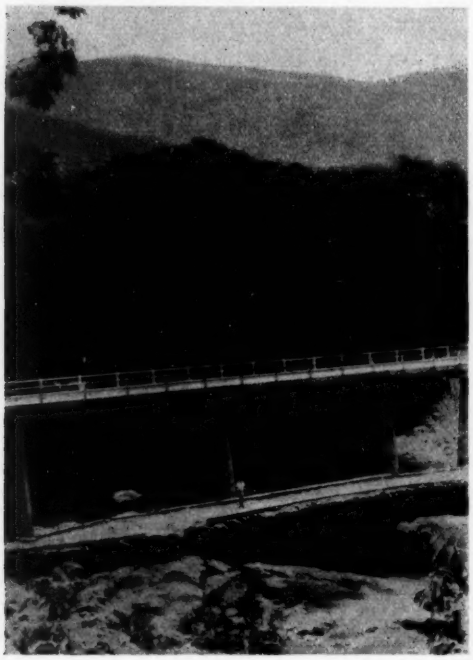

FIGURE IX

Bridge over the Tully River. Four of the men employed on its construction developed leptospirosis. A previous temporary bridge is also to be seen.

The cane areas are intersected by tramways—light railways—which serve to transport cane to the mills. A bridge carpenter in the Gordonvale district worked in water to repair and clear flood debris from a tramway bridge and suffered a *hyos* infection. A few days later another tramway employee in the same area also became infected with *hyos*.

Miners

Tin mining at Cooktown is alluvial. The ore-containing clay is excavated or washed out by a stream of water forced through a nozzle. It is washed or carried into a wooden " race ", which is built in a series of steps running down hill. The heavy grains of tin ore concentrate in the upper levels, while the clay is washed down to discolour the rivers of the district. The men work in bare feet, directing the stream of water, turning over the soil to keep the lighter particles moving and picking out debris from the race (Figure X). The water supply may be derived from a stream at a higher level or pumped up from a dam by a motor. The methods seem admirably adapted to catch any leptospiræ that may be there.

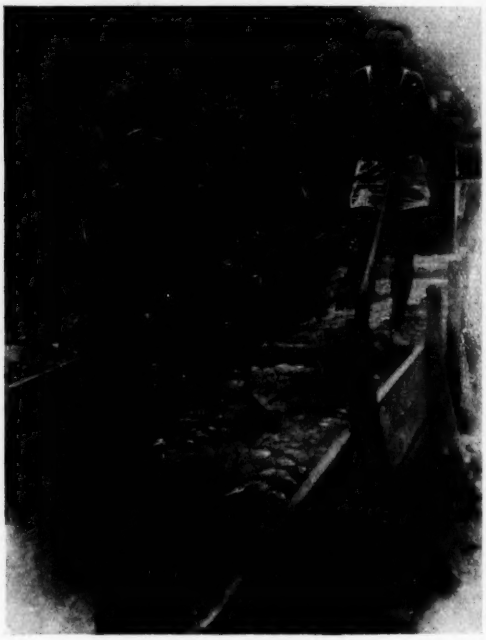

FIGURE X

Alluvial tin mining in the Cooktown district. Leptospiral infection is encouraged by working in water barefooted with its risk of injury on the stony rubble.

Three types of leptospiræ (*canicola*, *australis B* and " Celledoni ") were represented among the three tin miners.

Two wolfram miners with *hyos* infections from Mount Perseverance have already been mentioned.

Non-Occupational Leptospirosis

Although leptospirosis was thus often associated with particular occupations, there were other cases in which the infection was due

TABLE VIII
Leptospiral Type and Occupation

Type of Leptospira	Canefield Workers	Workers on Other Farms	Timber-getters and Scrub Clearers	Bridge and Tramway Workers	Miners	Various	Schoolboys and Schoolgirls	Females, other than Schoolgirls	Total
Icterohæmorrhagiæ ..	1	—	2	—	—	—	1	—	4
Canicola	2	1	—	—	1	1 unemployed	2	1	8
Australis A	26	—	1	—	—	2 sugarmill workers } 3 1 teacher	—	—	30
Australis B	18	1	3	1	1	3 sugarmill workers 1 sawmill worker } 7 1 butcher 1 fisherman 1 unemployed	4 (2 girls)	—	35
"Robinson" ..	7	—	1	2	—	1 breadcarter	1	—	12
Pomona	2	—	—	—	—	—	—	—	2
Hyos	5	—	—	2	2	—	2	2	13
Medanensis	2	—	—	—	—	—	—	—	2
"Kremastos" ..	11	—	2	1	—	1 mechanic	4	1	20
"Szwajizak" ..	6	—	—	—	—	1 laundryman	—	—	7
"Celledoni"	5	2	1	—	1	1 herdsman	1	—	11
Undetermined ..	2	2	1	1	—	1 salesman	1	—	8
Total ..	87	6	11	7	5	16	16	4	152

to activities unrelated to occupation. The teacher was probably infected with *australis A* while fishing and shooting along the Russell River. Shooting, fishing or swimming had taken others into jungle, swamp or river. Six patients, including two sugarmill employees, were spear-fishing in fresh water shortly before their illness. The herdsman and sawmill employee were addicted to the shooting of wild pigs. Most of the children gave a history of swimming, or of playing on the bank of a creek. The salesman had cleaned out a sump and drain at his residence.

The exact significance of a history of swimming is difficult to assess. Swimming is an extremely common activity and must have occurred many times with our patients when it was not mentioned in the history, as well as many times among the much larger population who remained well.

Occupation in Relation to Leptospiral Serotype

The only significant association of a particular type with occupation (Table VIII) was that of *australis A* with canefields. Canefield workers accounted for 87% of the *australis A* cases, compared with 57% of the total cases ($\chi^2=11\cdot8$, $P<0\cdot001$). Of the other four attacked by *australis A*, the teacher and a timber getter became infected at places close to canefarms. However, several of the canefield workers, as well as a sugarmill employee, may have been infected while spear-fishing.

Serological evidence, already mentioned, suggests that *australis A* has a much wider distribution in Australia than the canefields; it also occurs overseas. However, it would appear that at times the canefields provide conditions highly favourable for its transfer to man.

Australis B provides a contrast to the selectivity of *australis A*, for it is represented in every group in Table VIII except the older females. "Kremastos", *hyos*, *canicola*, "Robinson" and "Celledoni" were also associated with a wide range of occupations.

Although all seven "Szwajizak" patients had canefield associations (the laundryman lived on a canefarm), it has previously been noted that a bandicoot carrying "Szwajizak" agglutinins came from Daintree, at least ten miles from a canefield. Similarly there is evidence of *medanensis* at Daintree. The serum of a girl, aged six years, living on a dairyfarm there and convalescent after an acute febrile illness, agglutinated *medanensis* to a titre of 1 : 1000. The possum, the serum of which agglutinated *medanensis*, provides further evidence that this type may exist remote from canefields.

While most *australis A* infections were associated with canefields, the converse did not hold; 70% of the 87 canefield workers had other than *australis A* infections, and indeed every Queensland leptospiral type was represented among them. Among the other

occupations also, the types were widely represented. There were six types among 11 timber getters and scrub clearers, four among the seven workers in the bridge and tramway group, and seven types among 16 scholars. Among 12 who gave a recent history of swimming there were seven types, and three types attacked the six spear-fishermen.

DISCUSSION

Salient aspects in the epidemiology of leptospirosis are the animal sources of infection, association with water, and occupation.

Animal investigations are not dealt with in this paper, as we have, as yet, few observations to offer. With six of our leptospiral types, important animal sources of infection are already known from work in Australia or overseas—*Rattus norvegicus* for *icterohæmorrhagiæ*, *Rattus conatus* Thomas for *australis A* and *australis B*, the dog for *canicola*, and the pig and cow for *pomona* and *hyos*. *Medanensis* has been isolated from a dog in Sumatra. The remaining four North Queensland types have not yet been isolated from animals.

Examples are recorded in the literature of direct infection of man from a carrier animal, and there is no reason to doubt that this may occur on occasion in North Queensland. But in general our studies emphasize the importance of contact with water as an intermediary between animal and man. The endemic area is particularly well watered, and outbreaks are liable to follow heavy rain ; most of the probable sites of infection were adjacent to rivers or creeks ; many patients had been working or sporting in water.

It would appear that occupation, or recreation, is significant in the transmission of leptospirosis largely to the extent that it brings the patient into contact with infected water. Contact with water may in some cases be an essential accompaniment of the work, in others it may be incidental to it, or have occurred outside working hours.

How and when the waters became infected are matters for further investigation. Most waters in North Queensland, like the soils, are acid. Of 126 samples of running water tested in June, 1953, the pH of 75% lay between 5·7 and 6·8. The variation in incidence from place to place provides a further problem. Is the incidence influenced by the kind of soil ? Does the small number of recorded cases in some areas represent a genuinely low incidence or incomplete recording ?

The recent realization that as many as eleven leptospiral serotypes cause infection in North Queensland has much increased the complexity of the epidemiological picture. However, a study of the behaviour of the different leptospiral types shows more similarities among them than differences. Each one about which we have a reasonable body of evidence is widespread geographically, although some types had preferences for certain localities. In each of the occupational and age groups there was a wide variety of leptospiral types represented, although there were minor differences and, in particular, *australis A* was prone to attack canefield workers. The original animal sources of the leptospiral types may prove, when known, to be diverse, but the types seem to behave much alike when they reach the immediate external environment of man.

SUMMARY

Leptospirosis is endemic in a narrow, well-watered, coastal belt of North Queensland, extending from Ingham nearly to Cooktown. In this area, it is the commonest indigenous fever.

By intensive investigations during the two years 1951 to 1953, 152 cases have been recognized. They were caused by 11 serotypes of leptospira, the commonest being *australis B*, *australis A* and " Kremastos ".

Geographical analysis showed a patchy distribution. Cases tended to arise in the vicinity of rivers and creeks and were much commoner in some areas than in others.

A noteworthy focus of infection was an area which borders Babinda Creek for a distance of about four miles and includes the township of Babinda. Here at least 19 patients were infected.

Among other areas, cases occurred near Mossman, and it appears that leptospirosis is a major constituent of " Mossman fever ".

In a locality favourable to leptospirosis, there is likely to exist a profusion of leptospiral types.

Cases of leptospirosis in Johnstone shire showed a striking correlation with the rainfall eight to eighteen days before the onset, particularly at twelve days. With cases in Division 3 of Mulgrave shire, the association with wet weather was not so pronounced, but there was significant correlation with rain ten and eleven days before. As the incubation period of leptospirosis, as found by Schüffner, is four to nineteen days with an average of ten days, there is a rapid development after rain of conditions favouring human infection.

In the exceptional cases of leptospirosis occurring in dry weather, which are more common in Mulgrave 3 than in Johnstone, a history of contact with water from spear-fishing, swimming or cleaning drains could sometimes be elicited.

The ages of the patients ranged from six to sixty-eight years. Most cases (89%) were in men in the working years. Only six were in females.

As regards occupation, 57% of cases occurred in canefield workers, and all 11 leptospiral types were represented among them. Smaller groups comprised schoolboys, timber getters and scrub clearers, bridge and tramway workers and miners.

Twenty-six of the 30 *australis A* infections occurred in canefield workers. However, animal studies show that the geographical distribution of *australis A* extends far beyond the canefields. No other leptospiral type had a significant association with a particular occupation.

ACKNOWLEDGEMENTS

We are indebted to the medical practitioners of North Queensland for their cooperation in these studies, to Mr. B. W. Newman, Deputy Director of the Meteorological Branch, and his staff for rainfall records, to Mr. P. B. McGovern, M.A., B.Sc., for statistical advice, and to Mrs. W. Powell, Mr. E. W. Hollywood and Miss L. Pegus for assistance in preparing the illustrations.

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THE SEROLOGICAL CLASSIFICATION OF 89 STRAINS OF LEPTOSPIRÆ FROM NORTH QUEENSLAND, INCLUDING FIVE SEROTYPES NEW TO AUSTRALIA[1]

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Although leptospirosis was first diagnosed in Australia at Ingham in North Queensland in 1933 (Morrissey, 1934; Cotter and Sawers, 1934), up to 1951 only five types of leptospiræ pathogenic for man were known to occur in this country. These were *Leptospira australis A*, *L. australis B*, *L. icterohæmorrhagiæ*, *L. pomona* and *L. mitis* Johnson.[2] Of these five types only *L. australis A*, *L. australis B* and *L. mitis* Johnson have been proven by culture as occurring in North Queensland.

For many years sera have been submitted to the Laboratory of Micro-Biology and Pathology from fever patients in North Queensland, and agglutination tests have been carried out with the five strains known to occur in Australia. Opportunities to examine cultures were few, and no real advance was made in our knowledge of leptospirosis in North Queensland until the Field Station of the Queensland Institute of Medical Research was established at Innisfail in May, 1951. Since that time a detailed study has been made of as many fever patients as possible, and it very soon became evident that leptospirosis as it occurred in North Queensland was not only a major problem, but a more complicated one than had previously been realized.

Cultures are made whenever possible from patients suspected of having leptospirosis, and once leptospiræ have been demonstrated the cultures, together with paired sera, are sent to the Laboratory of Micro-Biology and Pathology. During the last twenty-eight months 89 cultures of leptospiræ have been investigated, and the results of the serological typing of these strains provided the material for this paper.

Methods

Of the 89 cultures of leptospiræ in this series, 88 were isolated from patients and one from the kidney of a rat, *Rattus conatus* Thomas. All were obtained in North Queensland by the staff of the Field Station of the Queensland Institute of Medical Research situated at Innisfail. The primary isolation of two strains was made by the inoculation of the patient's blood into guinea-pigs; the remainder were isolated by blood culture. Blood was obtained by venepuncture, and one millilitre was introduced immediately into both Schüffner's and Fletcher's media. Recently if the patient had received penicillin before the blood was collected, penicillinase, sufficient to inactivate 200 units per cubic centimetre, was added to the culture. The cultures were incubated at 37° C. but within recent months a temperature of 29° or 30° C. has been used. The cultures were examined by dark-ground illumination with the use of low-power objective on the seventh, fourteenth, twenty-first and twenty-eighth days of incubation. If the cultures showed no growth after this period, they were discarded; if, on the other hand, leptospiræ were demonstrated, subcultures were prepared and both the subcultures and the original cultures were forwarded by air to Brisbane for investigation.

At the Laboratory of Micro-Biology and Pathology, in Brisbane, the cultures were maintained in Schüffner's medium and incubated at 30° C. Subcultures were made weekly until the growth was suitable for use as an antigen. Agglutination tests were then carried out, the

[1] Received on February 11, 1954.

[2] It was decided by the International Committee for Nomenclature at its recent meeting in Rome to replace the name *L. mitis* Johnson by *L. hyos*, but this name is as yet not "official". At the present time *L. mitis* Johnson is still allowable, and it is retained in this paper since it is more familiar.

live culture being used as an antigen with type antisera which had previously been prepared in rabbits and which were known to be of a suitable titre (1 : 3000 or 1 : 10,000). For the agglutination test serial dilutions of the antisera were prepared on porcelain plates, and an equal volume of the culture was added. The plates were incubated for one and a half hours at 37° C. and were then read under dark-ground illumination with a low-power objective. Type antisera were stored at temperatures between −25° and −30° C. when not in use.

Antisera were prepared in rabbits from the cultures under investigation by the following technique.

A well grown Schüffner subculture was killed by exposure to a temperature of 56° C. for fifteen minutes, and one millilitre of this culture was injected into the marginal ear vein of a rabbit on the first day. This dosage was repeated on the fifth, eighth and twelfth days, and then on the fifteenth day one millilitre of the living culture was injected in a similar manner. Blood was obtained by heart puncture on the twenty-second day and the serum titrated, and if the titre was found to be 1 : 3000 or greater this was regarded as satisfactory for use. If this titre was not obtained the rabbit was bled again three or more days later and the serum again titrated.

The antisera so prepared were stored in the " deep freeze " in corked tubes. These antisera prepared from the cultures were then available for use in agglutination tests against the cultures of leptospiræ. The type cultures used in the laboratory are *L. icterohæmorrhagiæ* (Jackson), *L. australis A* (Ballico), *L. australis B* (Zanoni), *L. mitis* Johnson (Mackney), *L. pomona* (Staines), and *L. canicola* (Berlin).

RESULTS

In Table I are set out the results of the serological typing of the 89 cultures according to the serogroup to which they belong, and an attempt has been made to arrange them into their serotypes. Since no absorption tests were carried out the arrangement according to serotype was somewhat tentative, but our results have been confirmed by Dr. J. C. Broom, of the Wellcome Laboratories of Tropical Medicine, London, in all those cultures he has checked.

As a result of this investigation it is now known that there are 11 distinct leptospiral serotypes, pathogenic to man, occurring in North Queensland. It is noteworthy that of the 89 cultures, 36 (40%) belong to six serotypes which were not previously known to occur in Australia. These are *L. canicola*, *L. medanensis* and the " Robinson ", " Kremastos ", " Szwajizak " and " Celledoni " serotypes. From this series the frequency of occurrence of the various types of leptospiræ in North Queensland is as follows : *L. australis B* 24·7%, *L. australis A* 21·4%, " Kremastos " 15·7%, *L. mitis* Johnson 8·9%, *L. canicola* and " Robinson " 6·7%, " Szwajizak " and " Celledoni " 4·5%, and *L. medanensis*, *L. pomona* and *L. icterohæmorrhagiæ* 2·3%.

Serogroup Icterohæmorrhagiæ

L. icterohæmorrhagiæ was first isolated in Australia in Brisbane in February, 1937 (Johnson, Brown and Derrick). *L. icterohæmorrhagiæ* was represented by two cultures in this series, and this is the first report of the isolation by culture of this organism in North Queensland. Cross-reactions with *L. australis B* and *L. canicola* were obtained, with both the test cultures and the antisera prepared in rabbits from these cultures ; but the titres obtained left no doubt as to the identity of the organism in either case.

TABLE I

The Serotyping of 89 Cultures of Leptospiræ

Serogroup	Serotype	Number	Percentage
Icterohæmorrhagiæ	*L. icterohæmorrhagiæ*	2	2·3
Canicola	*L. canicola*	6	6·7
Australis A	*L. australis A*	19	21·4
Pyrogenes	*L. australis B*	22	24·7
	" Robinson "	6	6·7
Hyos (syn. *mitis* Johnson)	*L. mitis* (Johnson)	8	8·9
Pomona	*L. pomona*	2	2·3
Hebdomadis	*L. medanensis*	2	2·3
	" Kremastos "	14	15·7
	" Szwajizak "	4	4·5
?	" Celledoni "	4	4·5
Total	.. ..	89	—

Serogroup Canicola

In a previous communication (Sinnamon, Pask, Smith, Brown and Tonge, 1953) the isolation of *L. canicola* in Australia was reported, the two cases mentioned being part of this series. Since that time *L. canicola* has been isolated from four other patients in North Queensland.

The cultures were agglutinated by the type *L. canicola* antiserum to high titre, and the only other reaction noted was cross-agglutination with the " Robinson " strain in much lower titre than with the homologous antiserum. Antisera prepared from these six test cultures reacted with *L. canicola* in high titre in all but one, and in this the titre, though only 1 : 100, was the only reaction obtained. Two of the cultures were checked by absorption tests carried out by Dr. Broom and their identity was confirmed.

Serogroup Australis A

L. australis A, long known to be present in the canefields of North Queensland, was found to constitute 21·4% of the cultures. All the 19 cultures of *L. australis A* were agglutinated by the homologous type antiserum at least to full titre, and significant cross-reactions with heterologous strains were absent. The culture isolated from the rat was of this serotype.

L. australis A and *L. australis B* were first identified by Lumley in 1937 from strains previously isolated in North Queensland by Cotter and Sawers (1934).

Serogroup Pyrogenes

Of the pyrogenes serogroup two serotypes were found to be present in the culture series. Twenty-two of the cultures (24·7%) gave serological reactions of *L. australis B* serotype. On July 24, 1951, the "Robinson" strain was isolated, and it is representative of a group of six strains having close affinities to, but distinct from, *L. australis B*. We formed the opinion that these strains represented a distinct serotype within the pyrogenes serogroup, and this has since been confirmed by Broom (1953). The reactions of these six strains are set out in Tables II and III, and in these tables typical members of the *L. australis B* serotype are also included for comparison.

Our attention was first drawn to the possibility of a new serotype being present when it was found that the serum from the patient Robinson agglutinated in high titre the "Robinson" culture but failed to agglutinate *L. australis B*. The "Robinson" culture was agglutinated only to 10% of the titre of *L. australis B* antiserum and to 1% of the titre of the *L. canicola* antiserum. An antiserum prepared from the culture likewise agglutinated *L. australis B* only to 3% of its titre and *L. icterohæmorrhagiæ* to 1%.

It was apparent that the "Robinson" strain was not typical of *L. australis B*, and it was tentatively regarded as a distinct type and incorporated as a test organism for the investigation of further cultures. Five additional strains which resembled the "Robinson" strain were subsequently discovered. Sera from the five patients from whom these five strains were isolated agglutinated both the "Robinson" culture and their own cultures in high titre, but gave insignificant reactions with *L. australis B*.

These six strains of the "Robinson" type were forwarded to Dr. Broom for further investigation and he has recently reported that "they possess a specific antigen which differs from those of *L. pyrogenes* and *L. australis B*" (Broom, 1953).

This "Robinson" strain thus not only represents a new serotype for Australia, but appears to be an entirely new serotype of the pyrogenes group. Its ultimate status and nomenclature await determination.

Serogroup Hyos (Synonym Mitis Johnson)

L. mitis Johnson was originally isolated at Innisfail in June, 1940, and at that time represented the fifth type of leptospira to be

TABLE II

Titres of Type Antisera Against the Six "Robinson" Serotype and Five Typical L. australis B Cultures[1]

Cultures	Type Antisera										
	L. icterohæmorrhagiæ (3000)	*L. canicola* (10,000)	*L. australis A* (3000)	*L. australis B* (3000)	"Robinson" (3000)	*L. pomona* (10,000)	*L. mitis* Johnson (10,000)	*L. medanensis* (10,000)	"Kremastos" (10,000)	"Szwajizak" (3000)	"Celledoni" (10,000)
"Robinson" serotype:											
Rob.	0	100	0	300	3000	0	0	0	0	0	0
Bou.	10	100	0	1000	3000	0	0	0	0	0	0
Ang.	30	300	0	1000	10,000	0	0	0	0	0	0
Sor.	100	10	0	1000	3000	0	0	0	Not tested	Not tested	0
Del.	30	30	0	1000	3000	0	0	0	0	0	0
Con.	300	100	0	1000	10,000	0	0	0	Not tested	Not tested	0
L. australis B serotype:											
Dou.	30	1000	0	10,000	300	0	0	0	0	0	0
Lif.	300	300	0	10,000	1000	0	0	0	0	0	0
Bev.	1000	300	0	10,000	1000	0	0	0	0	0	0
Tan.	300	300	0	10,000	300	0	0	0	0	0	0
Jon.	100	300	0	10,000	100	0	0	0	0	0	0

[1] The figures shown are the reciprocal of the titres; "0", negative result in dilutions of 1 : 10 and upwards.

TABLE III

Titres of the Six "Robinson" and Five Typical L. australis B Antisera against Type Cultures[1]

Antisera	Culture										
	L. icterohæmorrhagiæ	*L. canicola*	*L. australis A*	*L. australis B*	"Robinson"	*L. pomona*	*L. mitis* Johnson	*L. medanensis*	"Kremastos"	"Szwajizak"	"Celledoni"
"Robinson" serotype:											
Rob. (3000)	10	0	0	100	3000	0	0	0	0	0	0
Bou. (10,000)	0	0	0	300	3000	0	0	0	0	0	0
Ang. (10,000)	30	0	0	3000	10,000	0	0	0	0	0	0
Sor. (3000)	10	10	0	100	3000	0	0	0	0	0	0
Del. (10,000)	0	0	0	1000	3000	0	0	0	0	0	0
Con. (3000)	0	0	0	100	1000	0	0	0	0	0	0
L. australis B serotype:											
Dou. (3000)	100	30	0	1000	10	0	0	0	0	0	0
Lif. (10,000)	3000	100	0	10,000	30	0	0	0	0	0	0
Bev. (3000)	100	10	0	1000	0	0	0	0	0	0	0
Tan. (3000)	300	0	0	3000	300	0	0	0	0	0	0
Jon. (30,000)	300	0	0	10,000	1000	0	0	0	0	0	0

[1] The figures shown are the reciprocal of the titres; "0", negative result in dilutions of 1 : 10 and upwards.

identified in Australia (Johnson, 1942). It is regarded as belonging to a distinct serogroup in the world classification of leptospiræ. Eight cultures of this type were found in our series. The cultures were found to react with the homologous type antiserum alone, and antisera prepared in rabbits from these cultures reacted to full titre with the type *L. mitis* Johnson culture. The identification of these strains thus presented no difficulty.

Serogroup Pomona

L. pomona was originally isolated in the country town of Pomona in southern Queensland in July, 1936 (Clayton, Derrick and Cilento, 1937). Its presence has long been suspected in North Queensland by the results of agglutination tests on patients' sera submitted from that area; but the two cultures belonging to this type in this series represent the first time this organism has definitely been isolated in North Queensland. Here again the reactions of both the culture and the antiserum were quite distinct, and the only "crossing" which was noted was a reaction of the antiserum with *L. australis A* in an insignificant titre.

Serogroup Hebdomadis

The *hebdomadis* serogroup of leptospiræ has not previously been found in Australia. In this series of 89 cultures it has been found that 20 (22·5%) belong to the *hebdomadis* serogroup, and that amongst these are representatives of three distinct yet closely related serotypes.

Our attention was first drawn to this group of leptospiræ by the isolation of cultures from each of two brothers, named Ives, on November 17, 1951, and December 29, 1951, respectively. These two cultures, which appeared to be identical, were not agglutinated by any of the antisera from strains then known to occur in Australia. After the isolation of the "Ives" strains, a series of cultures was isolated from patients in North Queensland which failed to react with any of the known Australian strains, but which were, with one exception, agglutinated by the "Ives" antiserum to 10% or less of the full titre (see Table IV). It was thus evident that we were dealing with a group of related strains quite distinct from the known Australian types.

A series of "box titrations" was made with some of these cultures and their antisera, and from the results obtained it was concluded that there were probably three distinct serotypes represented. The strains "Kremastos", isolated on January 22, 1952, and "Szwajizak", isolated on February 11, 1952, together with the original "Ives" strain, were selected as being representative of the three types, and they were used to serve as antigens and for the production of antisera in further investigations. Of the 20 strains falling within this distinct serogroup, there were two, the "Ives" strains, in the first sub-group, 14, represented by the "Kremastos" strain, in the second sub-group, and four in the third sub-group represented by the "Szwajizak" strain.

The serological reactions of six of these strains, two taken from each sub-group, are set out in Table VI. In this table the titres are expressed as percentages of the reciprocal

TABLE IV

Titres of Type Antisera against Hebdomadis Serogroup Cultures[1]

Culture	Type Antisera										
	L. icterohaemorrhagiæ (3000)	*L. canicola* (10,000)	*L. australis A* (3000)	*L. australis B* (3000)	" Robinson " (3000)	*L. pomona* (10,000)	*L. mitis* Johnson (10,000)	*L. medanensis* (10,000) " Ives 1 "	" Kremastos " (10,000)	" Szwajizak " (3000)	" Celledoni " (10,000)
Ives 1	0	0	0	0	0	0	0	10,000	100	30	0
Ives 2	0	0	0	0	0	0	0	10,000	300	30	0
Kre.	0	0	0	0	0	0	0	1000	10,000	100	0
Car.	0	0	0	0	0	0	0	1000	10,000	100	0
McC.	0	0	0	0	0	0	0	1000	10,000	30	0
God.	0	0	0	0	0	0	0	1000	10,000	30	0
Cam.	0	0	0	0	0	0	0	300	10,000	100	0
Col.	0	0	0	0	0	0	0	1000	10,000	30	0
McD.	0	0	0	0	0	0	0	1000	3000	10	0
Sil.	0	0	0	0	0	0	0	1000	30,000	100	0
Twi.	0	0	0	0	0	0	0	3000	30,000	300	0
Bam.	0	0	0	0	0	0	0	1000	10,000	300	0
Phi.	0	0	0	0	0	0	0	1000	10,000	1000	0
Squ.	0	0	0	0	0	0	0	1000	10,000	1000	0
Val.	0	0	0	0	0	0	0	300	3000	300	0
Leo.	0	0	0	0	0	0	0	1000	10,000	300	0
Szw.	0	0	0	0	0	0	0	300	1000	3000	0
Dow.	0	0	0	0	0	0	0	300	1000	3000	0
Poh.	0	0	0	0	0	0	0	100	300	10,000	0
Asc.	0	0	0	0	0	0	0	300	1000	10,000	0

[1] The figures shown are the reciprocal of the titres; " 0 ", negative result in dilutions of 1 : 10 and upwards.

for the homologous strain, and the differentiation into three distinct sub-groups is clearly seen.

These cultures were submitted to Dr. Broom for confirmation of our belief that several distinct serotypes were represented, and in a recent report (Broom, 1953) he states that he has identified the two " Ives " cultures in our first sub-group as *L. medanensis.* These cultures were also submitted to Professor J. W. Wolff,

TABLE V

Titres of Hebdomadis Group Antisera against Type Cultures[1]

Antiserum	Culture										
	L. icterohaemorrhagiæ	*L. canicola*	*L. australis A*	*L. australis B*	" Robinson "	*L. pomona*	*L. mitis* Johnson	*L. medanensis* " Ives 1 "	" Kremastos "	" Szwajizak "	" Celledoni "
Ives 1 (10,000)	10	0	0	0	0	0	0	10,000	1000	300	0
Ives 2 (10,000)	0	0	0	0	0	0	0	10,000	100	100	0
Kre. (10,000)	0	0	0	0	0	0	0	100	10,000	1000	0
Car. (3000)	0	0	0	0	0	0	0	30	3000	300	0
McC. (10,000)	0	0	0	0	0	0	0	100	10,000	1000	0
God. (3000)	0	0	0	0	0	0	0	100	3000	300	0
Cam. (10,000)	0	0	0	0	0	0	0	300	10,000	300	0
Col. (10,000)	0	0	0	0	0	0	0	1000	10,000	1000	0
McD. (10,000)	0	0	0	0	0	0	0	30	3000	300	0
Sil. (10,000)	0	0	0	0	0	0	0	100	10,000	300	0
Twi. (10,000)	0	0	0	0	0	0	0	30	10,000	1000	0
Bam. (3000)	0	0	0	0	0	0	0	100	3000	1000	0
Phil. (3000)	0	0	0	0	0	0	0	10	1000	300	0
Squ. (10,000)	0	0	0	0	0	0	0	100	10,000	1000	0
Val. (3000)	0	0	0	0	0	0	0	30	3000	100	0
Leo. (3000)	0	0	0	0	0	0	0	10	1000	30	0
Szw. (3000)	0	0	0	0	0	0	0	30	100	3000	0
Dow. (10,000)	0	0	0	0	0	0	0	100	300	10,000	0
Poh. (1000)	0	0	0	0	0	0	0	0	10	3000	0
Asc. (10,000)	0	0	0	0	0	0	0	30	1000	3000	0

[1] The figures shown are the reciprocal of the titres: " 0 ", negative result in dilutions of 1 : 10 and upwards.

TABLE VI

Differentiation with in the Hebdomadis Serogroup[1]

Immune Serum of	Titre Against "Ives 1"	Titre Against "Ives 2"	Titre Against "Kremastos"	Titre Against "Caruana"	Titre Against "Szwajizak"	Titre Against "Downing"
"Ives 1"	100%	100%	10%	10%	3%	3%
"Ives 2"	100%	100%	1%	1%	1%	0·3%
"Kremastos"	1%	3%	100%	100%	10%	10%
"Caruana"	1%	1%	100%	100%	10%	3%
"Szwajizak"	1%	1%	3%	3%	100%	100%
"Downing"	1%	0·3%	3%	3%	100%	100%

[1] Titres in percentages of reciprocal for the homologous strain.

of the Institute of Tropical Hygiene, Amsterdam, by Dr. Broom, and Professor Wolff has confirmed the identification of the "Ives" strain (Wolff, 1952). With regard to the other strains, Dr. Broom confirmed the fact that the "Kremastos" and "Szwajizak" type strains do in fact represent two distinct serotypes, and that these latter two serotypes may represent entirely new members of the *hebdomadis* serogroup. Their relationship to known world serotypes within this group has yet to be determined.

The "Celledoni" Type Strains

The "Celledoni" strain was isolated on January 28, 1952, and it failed to react with any of the known Australian types, with the "Robinson" strain, or with either of the two serotypes within the *hebdomadis* serogroup which we had then isolated. The strain appeared to us to be entirely new and was submitted to Dr. Broom for investigation. In a personal communication Dr. Broom stated that the "Celledoni" strain failed to react with over 30 of the species of leptospiræ in his collection. Its only reaction was in a titre of 1 : 100, a small fraction of the homologous titre, with *L. javanica*, a strain recovered from cats in Indonesia. Three further apparently identical strains have been found in our series of cultures, and their serological reactions with other strains now known to occur in Australia are set out in Tables VII and VIII.

The true classification of this strain has yet to be determined by further work, but it is certainly new to Australia and may well prove to be a new world entity.

DISCUSSION

Although by the examination of paired sera from patients it is possible, in most cases, to determine whether a patient is actually suffering from leptospirosis, the agglutination test on patients' sera is of limited value in deciding the type of leptospira actually causing the infection. These limitations are particularly evident in an area such as North Queensland, where many different types of leptospiræ pathogenic for man occur. It has occasionally been noted in cases proven by culture that heterologous titres may exceed those to the infecting type of leptospira. Anamnestic reactions may further prove misleading. It is only by obtaining cultures that the type of leptospira causing an infection can

TABLE VII

Titres of Type Antisera Against the Four "Celledoni" Serotype Cultures[1]

Cultures	Type Antisera										
	L. icterohæmorrhagiæ (3000)	*L. canicola* (10,000)	*L. australis A* (3000)	*L. australis B* (3000)	"Robinson" (3000)	*L. pomona* (10,000)	*L. mitis* Johnson (10,000)	*L. medanensis* (10,000)	"Kremastos" (10,000)	"Szwajizak" (3000)	"Celledoni" (10,000)
Cel. ..	0	0	0	0	0	0	0	0	0	0	10,000
Pic. ..	0	10	0	0	0	0	0	0	0	0	10,000
Har. ..	0	0	0	0	0	0	0	0	0	0	10,000
Agl. ..	0	30	0	0	0	0	0	0	0	0	30,000

[1] The figures shown are the reciprocal of the titres; "0", negative result in dilutions of 1 : 10 and upwards.

TABLE VIII

Titres of the Four "Celledoni" Serotype Antisera against Type Cultures[1]

Antisera	Culture										
	L. icterohaemorrhagiæ	*L. canicola*	*L. australis A*	*L. australis B*	"Robinson"	*L. pomona*	*L. mitis* Johnson	*L. medanensis*	"Kremastos"	"Szwajizak"	"Celledoni"
Cel. (10,000)	0	0	0	0	0	0	0	0	0	0	10,000
Pic. (10,000)	0	0	0	0	0	0	0	0	0	0	1000
Har. (3000)	10	0	0	0	0	0	0	0	0	0	3000
Agl. (10,000)	0	0	0	0	0	0	0	0	0	0	3000

[1] The figures shown are the reciprocal of the titres; "0", negative result in dilutions of 1 : 10 and upwards

with certainty be determined, and only by such means will the presence of new strains be detected.

The serological examination of the 89 cultures in this series has proved most profitable, and our knowledge of leptospirosis as it occurs in North Queensland has been developed enormously. *L. canicola* has been isolated for the first time in Australia. A new serotype of the pyrogenes serogroup, the "Robinson" strain, has been discovered. The *hebdomadis* serogroup of leptospiræ has been isolated, and of this serogroup there appear to be three distinct serotypes present. One of these has been identified by Dr. Broom and Professor Wolff as *L. medanensis*; the other two require further investigation before their relationship to other serotypes of the *hebdomadis* serogroup is known. The "Celledoni" type strain of leptospira has been isolated from four patients.

There are now known to be at least 11 distinct types of leptospiræ pathogenic for man occurring in North Queensland. These 11 strains are now used in our routine screening of cultures and sera.

The serology of leptospirosis as it occurs in Queensland is complex, and it would be of value for an Australasian leptospiral reference laboratory to be established. Such a reference laboratory could accumulate data regarding leptospirosis in Australia, undertake the typing of cultures from other centres not equipped to carry out this work, and maintain close contact with similar laboratories in other countries. Only by such means will our knowledge of this important disease be increased and the complicated serology and nomenclature of the various strains be clarified.

SUMMARY

Details of the preliminary serological classification of 89 strains of leptospiræ from North Queensland are set out, together with their frequency distribution.

L. canicola has been isolated from six patients.

A new serotype of the pyrogenes serogroup, the "Robinson" strain, has been isolated from six patients.

The presence of the *hebdomadis* serogroup of leptospiræ has been established as occurring in Australia, and three distinct serotypes of this serogroup have been isolated. One of these serotypes has been identified as *L. medanensis*.

A further new strain temporarily named "Celledoni" has been isolated from four patients. The designation of this strain has not been determined, but preliminary investigations suggest that it may be a new world entity.

There are now known to be at least 11 distinct types of leptospiræ pathogenic for man in North Queensland. Forty *per centum* of the cultures in this series were of types not known to occur in Australia prior to 1951.

The desirability of establishing an Australasian leptospiral reference laboratory is stressed.

ACKNOWLEDGEMENTS

We are extremely indebted to Dr. J. C. Broom, of the Wellcome Laboratories of Tropical Medicine, for his valuable advice and encouragement. He has confirmed the typing of many of our cultures, and has undertaken the highly complex and laborious task of determining the true designation of the new strains isolated in our series.

We are indebted to Professor J. W. Wolff, of the Institute of Tropical Hygiene, Amsterdam, for carrying out absorption tests and confirming the identification of the "Ives" strains.

Our thanks are due to the many practitioners in North Queensland who have made available their patients and generally cooperated in this investigation.

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THE DIAGNOSIS AND MANAGEMENT OF ACQUIRED HÆMOLYTIC ANÆMIA[1]

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DURING recent years, considerable advances have been made in our knowledge of the pathogenesis and ætiology of that group of anæmias in which the essential abnormality is a shortened life span of the red blood cell—the hæmolytic anæmias.

The following classifications modified from Dacie (1951) are based on the present state of this knowledge:

Pathogenetic Grouping of Hæmolytic Anæmia.

1. Intrinsic Origin (Congenital or Acquired Disorders): (*a*) due to increased sensitivity of the patient's corpuscles to the normal mechanisms of destruction; the fault lies in the cells themselves; (*b*) due to the development of an abnormal hæmolytic mechanism; the fault lies in the patient's plasma or tissues; the red cells are primarily normal, but may be secondarily altered.
2. Extrinsic Origin, due to the effects of drugs, chemicals or toxins, physical agents *et cetera*.

The more important examples of hæmolytic anæmias of intrinsic origin are listed in the two following lists.

Hæmolytic Anæmias due to Corpuscular Abnormalities

1. Hereditary spherocytic hæmolytic anæmia (acholuric jaundice).
2. Mediterranean anæmia (Cooley's anæmia).
3. Atypical congenital hæmolytic anæmias.
   A heterogeneous group of rare disorders, showing either various morphological abnormalities—for example, ovalocytosis, small irregular poikilocytes, siderocytes—or morphologically normal red cells.
4. Chronic hæmolytic anæmia with paroxysmal nocturnal hæmoglobinuria.

Hæmolytic Anæmias due to an Abnormal Hæmolytic Mechanism

A. Auto-antibodies present:
   (i) Acquired hæmolytic anæmia (auto-immune): (*a*) primary (idiopathic), (*b*) secondary (symptomatic);
   (ii) Hæmolytic anæmia following virus pneumonia;
   (iii) Chronic hæmolytic anæmia with high titre cold agglutinins ("cold agglutinin hæmoglobinuria with Raynaud's syndrome");
   (iv) Paroxysmal cold hæmoglobinuria;
   (v) Hæmolytic disease of the newborn.

B. Auto-antibodies not present:
   (i) Acquired hæmolytic anæmia;
   (ii) Hypersplenism: (*a*) primary (idiopathic), (*b*) secondary (symptomatic).

The present report, based on a study of 22 cases of auto-immune acquired hæmolytic anæmia, discusses the variation in the clinical picture and the natural history of the disease, the response to splenectomy and to treatment with steroid hormones, and the serological findings. The differential diagnosis of this group from the other types of hæmolytic anæmia due to an abnormal hæmolytic mechanism (those listed in the last list) is discussed briefly. In the subsequent text auto-immune acquired hæmolytic anæmia will be referred to as AHA.

CLINICAL FEATURES

The clinical features are set out in Table I.

Age and Sex Distribution

With the exception of Cases 15 and 18, all patients were examined in adult hospitals; the study, therefore, does not cover the condition of AHA as it occurs in children. Of the adult females 50% were post-menopausal in the sixth and seventh decades; the ages of the other adults ranged from twenty-six to fifty-four years. All but one of the 22 patients were females.

Onset

In all except Case 6, in which an acute hæmolytic episode followed the administration of phenylbutazone, the onset was insidious, symptoms having been present usually over a period of months, occasionally over several weeks. Many patients complained of loss of appetite and some of definite loss of weight; these constitutional symptoms were as frequent in the idiopathic as in the symptomatic group, and therefore were of little value in indicating an underlying primary disease. Of the 22 cases, 10 were symptomatic (Table I). The frequent complaint of painful swollen joints, associated with either *lupus erythematosus* or rheumatoid arthritis, was a feature of this series. In two of these symptomatic cases the patients presented with AHA, the underlying disease

[1] Received on February 2, 1954.

[2] Aided by a grant from the National Health and Medical Research Council of Australia.

being discovered at post-mortem examination (Cases 1 and 20). The primary disease was apparent before the onset of the AHA in the other eight cases. Upper respiratory tract infection preceded the initial onset of anæmia in one case and relapses in two cases. Six patients had an associated urinary infection, two had staphylococcal skin infections, and one bronchiectasis. One patient (Case 7) relapsed after a pulmonary infection.

Physical Findings

Although clinical jaundice was present in the majority of cases, it was usually mild and obvious only in the sclera. In six cases icterus was persistently absent; four of these patients had serum bilirubin levels below 1·0 milligramme per 100 millilitres. The spleen was clinically palpable and slightly to moderately enlarged in all but two patients; one was a somewhat obese patient who had been examined at regular intervals for five years, and the other the patient with an ovarian dermoid cyst in whom the spleen was found to be of normal size at post-mortem examination. In both the cases in which AHA was secondary to malignant disease the spleen was palpable. Hepatomegaly was present in all but six patients. Lymphadenopathy was present only in some of the symptomatic cases, and was due to the underlying disease. Pyrexia was observed in most of the secondary cases and in some of the more severe idiopathic cases. No patient showed ulceration of the legs.

Hæmatology, Serology and Biochemistry

In the phase of active hæmolysis, in all cases anæmia with reticulocytosis was present, and in 17 cases there were found spherocytosis and an increase in osmotic fragility when it was determined quantitatively. Spherocytosis was usually of slight to moderate degree, although occasionally marked; when slight spherocytosis was present, the increase in fragility could be demonstrated only by the quantitative osmotic fragility test (Dacie, 1950) and not by the qualitative test. After splenectomy siderocytes appeared in the peripheral blood in three of seven subjects examined; they were absent in four who did not undergo splenectomy. A normal or moderately elevated white cell count was the rule, but persistent neutropenia was present in Case 11 (Felty's syndrome). Thrombocytopenia was present in five cases. Two patients (Cases 7 and 8) had previously had their spleens removed for thrombocytopenic purpura, which was the presenting symptom of their underlying disorder—*lupus erythematosus*.

Macroscopical granularity of the blood, due to the tendency of the sensitized cells to clump, was frequently noted at room temperature in the blood taken into oxalate for routine counts. This phenomenon, together with auto-agglutination in the blood film, first drew attention to the hæmolytic nature of the anæmia in two cases.

The direct Coombs test invariably gave a positive result at some stage of the disease in 20 patients examined. In Cases 1 and 11 signs of active hæmolysis were present for two and three weeks respectively before the Coombs test result became positive; in Case 6, after the administration of phenylbutazone, there was a persistently negative result to the Coombs test for one month before it became positive. Except for Case 19, the result of the Coombs test remained positive after splenectomy, irrespective of the clinical result (Table III). In Case 11, in which, as a result of steroid therapy, there was a negative result to the Coombs test before splenectomy, a positive result to the Coombs test developed again fifteen months after a sustained clinical remission. Steroid hormone therapy did not usually cause reversal of the Coombs test result, although the titre fell in some cases. In Cases 11 and 15 the Coombs test result became negative—in the former with, and in the latter without, clinical remission. There was no relationship between the titre revealed by the Coombs test and the degree of anæmia. Several severely anæmic patients had positive Coombs test results of low titre, while in Case 3, in complete remission, the Coombs titre was 1/1024.

The incidence of warm agglutinins, warm hæmolysins and cold agglutinins in the serum is recorded in Table II. Techniques used for their demonstration are described elsewhere (Dacie and de Gruchy, 1951).

The result of the Wassermann test was positive in four of 16 cases. There was no clinical evidence of syphilis in any patient, nor at post-mortem examination in Case 17.

The cephalin flocculation test result was positive and the blood sedimentation rate raised in all active cases, though both tests gave normal results in four cases during remission following either splenectomy or steroid hormone therapy.

Erythrophagocytosis was demonstrated in five active cases and was absent in three—one active and two during remission. The technique used was that of Zinkham and Diamond (1952), in which the buffy coat of the

TABLE I

Clinical Findings in 22 Cases of Acquired Hæmolytic Anæmia

Case Number	Patient's Age (Years)	Sex	Presentation	Ætiology	Associated Infections	Clinical Course	Known Duration of Illness
1	62	F.	Anæmia	Symptomatic. Primary carcinoma of the liver	Urinary infection.	No response to SHT.[1] Death from cerebral thrombosis. Carcinoma of the liver discovered *post mortem*	5 months
2	52	F.	Anæmia	Idiopathic	Nil	Partial remission after ACTH and blood transfusion. No response to cortisone. Clinically well, but condition still active	16 months
3	62	F.	Anæmia	Idiopathic	Urinary infection. Relapse after upper respiratory tract infection	Partial remission after splenectomy. Complete sustained remission after ACTH. Coombs test result still strongly positive	21 months
4	61	F.	Anæmia	Idiopathic	Urinary infection	Leads active life with hæmoglobin values 7·0 to 9·5 grammes *per centum*. Blood transfusion (4 pints) in 1951. Refuses other treatments. Spleen not palpable	5 years
5	64	F.	Lympathadenopathy. Anæmia developed subsequently	Symptomatic. Reticulosarcoma	Nil	No response to SHT. Death from reticulosarcoma. Auto-agglutination first indication of AHA	2 months
6	61	F.	Anæmia. Acute onset.	Symptomatic. Followed phenylbutazone therapy for rheumatoid arthritis	Relapse after upper respiratory tract infection	Partial response to SHT. No response to splenectomy. Now in relapse uncontrolled by SHT	9 months
7	38	F.	*Lupus erythematosus.* Anæmia developed subsequently	Symptomatic. *Lupus erythematosus*; L.E. cells present	Relapse after pulmonary infection	Splenectomy for thrombocytopenic purpura four months before onset of AHA. In remission after ACTH and blood transfusions	5 months
8	39	F.	*Lupus erythematosus.* Anæmia developed subsequently	Symptomatic. *Lupus erythematosus*; L.E. cells present	Staphylococcal skin infection	Splenectomy for thrombocytopenic purpura in 1948. Section of spleen suggested lupus. AHA developed in 1953. Partial remission after ACTH. No response to cortisone	8 months
9	64	F.	Anæmia	Idiopathic	Bronchiectasis	Partial sustained remission after ACTH	6 months
10	37	F.	Anæmia	Idiopathic	Nil	No response to splenectomy. Partial sustained remission after ACTH	27 months
11	68	F.	Felty's syndrome. Anæmia developed subsequently	Symptomatic. Rheumatoid arthritis. Felty's syndrome	Urinary infection	No response to SHT, though Coombs test result became negative. Sustained complete remission after splenectomy	18 months
12	26	F.	Anæmia	Idiopathic	Nil	Partial remission after ACTH. Relapse in January, 1954	18 months
13	52	F.	Anæmia	Idiopathic	Staphylococcal skin infection	Complete sustained remission after splenectomy	38 months
14	38	F.	Joint swellings. Anæmia developed subsequently	Symptomatic. Probably *lupus erythematosus*	Urinary infection.	No response to SHT. Partial remission after splenectomy. Died in acute relapse.	9 months
15	11	F.	Anæmia	Idiopathic	Nil	No response to splenectomy or cortisone. Sustained complete remission after ACTH	33 months
16	28	F.	Rheumatoid arthritis. Anæmia developed subsequently	Symptomatic. Rheumatoid arthritis	Nil	No response to splenectomy or ACTH. Partial remission after cortisone	6 months
17	58	F.	Anæmia	Idiopathic	Urinary infection	Complete sustained remission after splenectomy. Death six months later from coronary occlusion	15 months

[1] SHT (steroid hormone therapy) indicates course of both ACTH and cortisone.

TABLE I—*Continued*

TABLE I—*Continued*

Clinical Findings in 22 Cases of Acquired Hæmolytic Anæmia—Continued

Case Number	Patient's Age (Years)	Sex	Presentation	Ætiology	Associated Infections	Clinical Course	Known Duration of Illness
18	13	F.	Anæmia	Idiopathic	Nil	No response to splenectomy or exsanguination transfusion. Spontaneous remission for three months after 22·5 litres of blood had been given over a period of eight weeks. Death from rupture of old hæmatoma at splenectomy site	5 months
19	44	F.	Anæmia	Idiopathic	Nil	Complete sustained remission after splenectomy	17 months
20	61	F.	Anæmia	Symptomatic. Ovarian dermoid cyst	Nil	Died after transfusion reaction. Post-mortem examination revealed first evidence of ovarian dermoid cyst. Spleen normal size at post-mortem examination	9 months
21	52	M.	Anæmia	Idiopathic	Nil	Complete sustained remission after splenectomy	19 months
22	58	F.	Anæmia	Symptomatic. Clinically, classical *lupus erythematosus*. L.E. cells not found (one test only)	Nil	Partial sustained remission after ACTH	31 months

blood is incubated *in vitro*, and the smears from this are examined for phagocytosis of red cells by leucocytes.

RESULTS OF TREATMENT

Splenectomy was performed on 12 patients during the phase of active hæmolysis. Five had complete remissions lasting between nine and thirty-three months without relapse, two had short partial remissions, and five showed no clinical response. Of the "failed splenectomies", in three cases the AHA was idiopathic and in two symptomatic; of the successful cases, in four the condition was idiopathic and in one symptomatic (Table III).

Splenectomy did not cause reduction in antibody titre or a reversal of the Coombs test result except in Case 13. There was no relation between the clinical severity, antibody titre or serum complement level, and the likelihood of response to splenectomy.

Fifteen patients were treated with steroid hormones, ten with both ACTH and cortisone, and five with ACTH only. Of those treated with both, four responded to ACTH but not to cortisone, one to cortisone but not to ACTH, one to both, and four to neither. All those treated with ACTH alone showed some response. Complete sustained remissions occurred in Cases 3 and 15 for periods of eighteen and twenty-four months respectively without maintenance therapy. Partial remissions were obtained in nine patients, some of whom had small doses of cortisone as maintenance therapy. In four symptomatic cases (Cases 1, 5, 11 and 14), the patients responded to neither hormone. Doses and duration of treatment varied. ACTH was usually administered intramuscularly, but occasionally intravenously. 20 units in a litre of glucose solution being given over eight hours. Intramuscular doses averaged 50 to 100 milligrammes per day of ACTH and 100 to 200 milligrammes per day of cortisone. Duration of the courses varied from one week to eight weeks. In general the doses given were not so large as those recommended by Dameshek (1952).

Venous thrombosis occurred in two patients who were receiving steroid hormone therapy; one (Case 1) died of a cerebral thrombosis, and the other (Case 12) developed an axillary vein thrombosis which resulted in amputation of the arm.

Supportive blood transfusions were administered in a number of cases. One patient (Case 20) died after a transfusion reaction. Exsanguination transfusion was performed without effect in Case 18.

Discussion

Diagnosis

The diagnosis may be suspected from the clinical features, but must be confirmed by the hæmatological and serological findings. Failure to realize that jaundice is often absent may lead to the hæmolytic nature of the anæmia being overlooked. The auto-immune basis is demonstrated by the positive result to the Coombs test and by other serological findings (Dacie and de Gruchy, 1951).

In every case of AHA an underlying disease should be sought. Whilst this disease may manifest itself clinically later in the course of AHA, it may be revealed only at post-mortem examination, or when, after splenectomy, examination of sections of the spleen reveals specific pathological changes—for example, of Hodgkin's disease.

It is now well recognized that AHA may be the presenting symptom of *lupus erythematosus*, and that AHA may occur during the course of the established disease. Therefore, the L.E.

Table II

Laboratory Findings in 21 Cases of Acquired Hæmolytic Anæmia

Case No.	Coombs Test Result	Warm Agglutinins[1]	Warm Hæmolysins[1]	Cold Agglutinins[1]	Wassermann Test Result	Blood Sedimentation Rate (Millimetres in One Hour)	Cephalin Flocculation Test Result	Erythrophagocytosis	Serum Bilirubin Level (Milligrammes per 100 Mils)	L.E. Cells
1	Positive. Negative for first two weeks	64	0	2048	Negative	66	Positive	—	0·8	—
2	Positive	64	0	128	Negative	117	Positive	Present	1·4	Not found
3	Positive	8	0	4	Positive	4 (Remission)	Positive	—	2·5	—
4	Positive	16	0	4	Negative	80	Positive	Not present	1·6	Not found
5	Positive	0	512	512	Negative	148	Positive	—	2·5	—
6	Positive. Negative for first month	8	0	0	Negative	112 (Active) 4 (Remission)	Positive	Present	2·2	Not found
7	Positive	4	512 Disappeared during remission	64	Negative	106 (Active) 5 (Remission)	Positive	Present	1·5	Present
8	Positive	32	0	0	Negative	124	Positive	Present	1·8	Present
9	Positive	8	0	16	Negative	35	Positive	Present	0·8	Not found
10	Positive	64	0	0	Negative	100	Positive	—	3·0	—
11	Positive. Negative after ACTH	32	0	512	Negative	58	Positive	Not present (Remission)	0·7	Not found
12	Positive	4	0	16	Positive	110	Positive	Present	3·0	Not found
13	Positive	—	—	—	Negative	12 (Remission)	Negative (Remission)	Not present (Remission)	2·1	Not found (Remission)
14	Positive	32	0	—	Negative	129	Positive	—	1·3	Not found (One test only)
15	Positive	8	0	32	—	—	—	—	1·75	—
16	Positive	4	0	4	—	—	Positive	—	3·0	—
17	Positive	—	—	—	Positive	—	Positive	—	1·6	—
18	Positive. Negative during spontaneous remission	—	—	—	—	—	—	—	1·5	—
19	Positive	—	—	—	Negative	—	—	—	1·9	—
21	Positive	—	—	—	—	—	—	—	0·9	—
22	Positive	—	—	—	Positive	—	—	—	1·8	—

[1] The agglutinin and hæmolysin titrations are expressed as the reciprocal of the greatest dilution in which macroscopic agglutination is present on the one hand and in which macroscopic hæmolysis is visible on the other.

cell test should be performed as a routine procedure in all cases of AHA, and other clinical manifestations of lupus should be sought. If systemic lupus is suspected and no L.E. cells are found, the test should be repeated if necessary on a number of occasions at intervals of several weeks. In Case 7 many negative results were obtained before L.E. cells were found. Further, if the peripheral blood test gives negative findings, the bone marrow should be examined, as occasionally the L.E. cell can be demonstrated there and not in the peripheral blood (Dubois, 1953*a*). Whenever possible the test should be performed before steroid therapy is commenced, as Dubois (1953*b*) has found that with adequate therapy the L.E. cell test result reverted to negative in four to six weeks in cases in which a remission was successfully induced. As " false positive " results to the Wassermann test occur in both AHA and systemic lupus, the L.E. phenomenon should be sought, especially in those cases of AHA in which the Wassermann test produces a positive result.

More positive results to L.E. cell tests have been obtained since the defibrinated blood technique described by Morten and Blackburn (1953) has been used.

In addition to the diseases described in this series—*lupus erythematosus*, reticulosarcoma, rheumatoid arthritis, carcinoma, dermoid cyst—AHA may occur secondarily to lymphatic leuchæmia, lymphosarcoma, Hodgkin's disease, *periarteritis nodosa*, Boeck's sarcoidosis, and cirrhosis of the liver.

The fact that a " false positive " result to the Wassermann or Kahn test may occur in AHA is not generally recognized. Syphilis does not cause this type of hæmolytic anæmia. If there is clinical cause to suspect syphilis, the treponemal immobilization test (Beerman, 1953) should be performed.

A positive result to the cephalin flocculation test may lead to the erroneous diagnosis of a primary liver disease with secondary hæmolytic anæmia—this does occur, but is uncommon (Hyman and Southworth, 1951). The positive response to the cephalin flocculation test and

TABLE III

Results of Splenectomy in 12 Cases of Acquired Hœmolytic Anœmia

Case Number	Ætiology	Clinical Result	Laboratory Findings		
			Hæmoglobin Value (Grammes *per Centum*)	Reticulocytes	Coombs Test Result
3	Idiopathic	Partial remission for one month. Relapse following upper respiratory tract infection. Subsequent remission affer ACTH	Before: 6·0 After: 12·4	37·0% —	Positive Positive
6	Symptomatic. Followed phenylbutazone therapy for rheumatoid arthritis	No effect	Before: 4·6 After: 5·5	9·1% 5·8%	Positive Positive
10	Idiopathic	No effect	Before: 8·4 After: 6·0	6·0% 26·0%	Positive Positive
11	Symptomatic. Felty's syndrome	Complete remission sustained for 15 months	Before: 9·2 After: 15·7	4·8% 2·9%	Negative (after ACTH therapy) Positive
13	Idiopathic	Complete remission sustained for 33 months	Before: 7·4 After: 14·7	20·0% 2·2%	Positive Negative
14	Symptomatic. Probably *lupus erythematosus*	Partial remission for four months after splenectomy. Died in acute relapse	Before: 4·5 After: 9·2	6·0% 2·0%	Positive Positive
15	Idiopathic	No effect. Subsequent sustained complete remission affer ACTH	Before: 3·9 After: 6·5	6·2% 19·0%	Positive Positive
16	Symptomatic. Rheumatoid arthritis	No effect. Subsequent partial remission after cortisone	Before: 7·0 After: 6·5	14·0% 10·0%	Positive Positive
17	Idiopathic	Complete remission sustained for six months. Death from coronary occlusion	Before: 6·0 After: 11·3	24·0% 3%	Positive Positive
18	Idiopathic	No effect	Before: 9·8 After: 3·6	14% —	Positive Positive
19	Idiopathic	Complete remission sustained for 15 months	Before: 10·0 After: 12·7	4% 2%	Positive Negative
21	Idiopathic	Complete remission sustained for 13 months	Before: 10·0 After: 13·0	7·0% 0·7%	Positive Positive

the raised sedimentation rate are due to the increase in serum γ globulin which occurs in active cases of AHA.

Differential Diagnosis.—The differentiation of acquired hæmolytic anæmia from hæmolytic anæmias due to corpuscular abnormalities is usually not difficult when studies of family and clinical histories and the appropriate special examinations are carried out. These are described in standard text-books. The main conditions which may be difficult to distinguish from acquired hæmolytic anæmia are those listed in the last of the three lists set out earlier.

Hæmolytic Anæmia Following Virus (Atypical) Pneumonia.—This is a rare complication of virus pneumonia, which usually occurs during convalescence. The onset is usually sudden, hæmoglobinuria may occur, the spleen may be palpable and the attack is self-limiting. The pattern of antibodies present in the serum differs from that of primary acquired hæmloytic anæmia (Dacie and de Gruchy, 1951); the direct Coombs test result is positive, but cold agglutinins are present in a much higher titre—for example, 2000 to 16,000—and cold hæmolysins are present. In acquired hæmolytic anæmia the titre of cold agglutinins when present is usually under 1000, although in Case 1 of this series it was 2000. These features should serve to distinguish this type of hæmolytic anæmia; however, it must be remembered that an exacerbation of acquired hæmolytic anæmia may follow pneumonia.

Chronic Hæmolytic Anæmia with High Titre Cold Agglutinins ("Cold Agglutinin Hæmoglobinuria with Raynaud's Syndrome").—The clinical picture is of a chronic hæmolytic anæmia affecting adults with Raynaud's syndrome. The anæmia is more severe in winter, when attacks of hæmoglobinuria are not infrequent. The direct Coombs test produces a positive result. Cold agglutinins are present in very high titres—for example, to 128,000—and cold hæmolysins of high thermal amplitude are present (Ferriman *et alii*, 1950).

Paroxysmal Cold Hæmoglobinuria.—This condition, usually though not invariably due to syphilis, often congenital, has been fully characterized both clinically and pathologically. The Donath-Landsteiner test is diagnostic.

Acquired Hæmolytic Anæmia.—Rare cases of acquired hæmolytic anæmia occur which show the usual evidences of excess blood destruction (icterus, raised serum bilirubin level, raised urinary and fæcal urobilinogen excretion) and of blood regeneration (reticulocytosis), but in which there are a persistently negative result to the Coombs test and no antibodies in the serum. These may be related to the condition of hypersplenism described below.

Hypersplenism. — This condition is characterized by the presence of an enlarged spleen, pancytopenia, an active bone marrow, and a well defined and sustained clinical remission following splenectomy. It appears that inhibition of marrow production plays the major part in the pathogenesis of the anæmia in these cases, but there is evidence of occult excess destruction, in that red cell survival time may be shortened when normal red cells are introduced into the circulation, and in that the fæcal urobilinogen excretion may be increased. Icterus and reticulocytosis are usually absent, though occasionally present. Hypersplenism is most frequently associated with splenomegaly secondary to some well defined disease—for example, portal hypertension, reticulosis—but in a small number of idiopathic cases no cause for the enlarged spleen can be demonstrated.

Management

Blood transfusion, the administration of steroid hormones and splenectomy are the sheet anchors of treatment.

Blood Transfusion.—Blood transfusion may not be required in mild cases, but should be used in severe cases whenever necessary to maintain a red cell count and blood volume compatible with life. In the AHA of adults, transfusions usually do not have the beneficial limiting effect on the hæmolytic process seen in the acute hæmolytic anæmia of children (so-called Lederer's anæmia), in which transfusions of fresh whole blood may be followed by a dramatic cessation of hæmolysis. However, in several cases of this series the rate of hæmolysis did seem to be definitely slowed after transfusion. As reactions to transfusion are not uncommon, in general transfusions should be kept to a minimum, especially if they appear to be ineffective. The blood should be fresh, and cross-matching should be carried out by the albumin and indirect Coombs techniques. Because of the tendency to auto-agglutination the blood may be incorrectly typed as AB, Rh-positive. If any suspicion about the blood group exists, the red cells should be thoroughly washed with normal saline at 37° C., and typed at 37° C. Reactions are usually due to the action of agglutinins in the patient's serum on the donor's cells. Dameshek and Neber (1950) have described a plasma reaction which is similar clinically to the ordinary incompatible transfusion reaction, but which lacks the evidences of intravascular hæmolysis—hæmoglobinæmia and hæmoglobinuria. These reac-

tions may be detected by the use of the " plasma provocative test ", in which 10 to 20 cubic centimetres of normal plasma are injected intravenously. If the result is positive, donor red cells washed three times with normal saline should be used; but if this is not feasible, packed red cells from which the plasma has been removed will usually be satisfactory unless the patient reacts to very small amounts of plasma. In the serum of between 10% and 20% of patients, a warm hæmolysin requiring the presence of complement can be demonstrated, in addition to a warm agglutinin. Although it is not certain that this in-vitro hæmolysin is a cause of hæmolysis *in vivo*, in such cases it is wise to give packed cells without plasma so that the complement content of the patient's plasma will not be increased.

Splenectomy.—The results of splenectomy in this series are in accord with those of Dameshek (1950), who in a series of 52 cases noted complete remissions in 50% of idiopathic cases and in one-third of the symptomatic cases. Except in Case 19, splenectomy did not cause a reduction in antibody titre or reversal of the Coombs test result, so that it seems that the beneficial effect of splenectomy is the result of the removal of the major site of destruction of sensitized cells, rather than the removal of an important site of antibody production. In cases in which the response is unsatisfactory, it appears that the rest of the reticulo-endothelial system is playing a large part in red cell destruction. In Case 6 (" failed splenectomy ") the liver was observed at operation to be very large, although the patient was not in cardiac failure.

It is not possible at present to predict from either the clinical or laboratory findings which patients will respond to splenectomy alone. It appears that splenectomy should now be reserved for patients with idiopathic AHA who have not responded to adequate steroid hormone therapy, and for those with symptomatic AHA who have not responded to treatment of the underlying condition or to treatment with steroid hormones. Splenectomy should also be considered for those patients who have responded to steroid hormone therapy, but in whom after some months there are still signs of activity, and who require large doses of hormones to maintain a reasonable hæmoglobin level; even if complete remission does not follow splenectomy, it is possible that an adequate hæmoglobin level may be maintained with smaller doses of hormone than were required before splenectomy.

At operation the abdomen should be explored for ovarian dermoid cysts, malignant tumours of the bowel, and enlarged glands. If they are resectable such tumours should be removed. Accessory spleens should also be searched for and removed.

Examination of sections of the spleen may give the first evidence of an underlying condition—for example, Hodgkin's disease, Boeck's sarcoidosis.

Steroid Hormone Therapy.—Steroid hormone therapy, either alone or combined with blood transfusion in more severe cases, is the initial treatment of choice. ACTH in doses of 150 to 300 milligrammes daily should be tried first. Improvement when it occurs is usually prompt, but maintenance treatment over varying periods of time may be required. Dameshek (1952) has stressed the necessity of adequate dosages over a sufficient length of time. If ACTH does not cause remission, then cortisone should be tried, as response may occur to one hormone and not to the other. When remission has been induced doses should be gradually lowered. Some patients remain in remission without any maintenance therapy; others may require small maintenance doses—for example, 50 milligrammes of cortisone per day by mouth (Letman, 1953)—whilst some require large maintenance doses; the question of splenectomy should be considered for the latter group. An increased incidence of thrombosis in patients under treatment with steroid hormones has been described by Cosgriff (1951). As anæmia in general, and acquired hæmolytic anæmia in particular, is a predisposing cause of thrombosis, patients with severe anæmia who do not show prompt response to steroid therapy should receive blood transfusions.

General Measures.—Since infection, especially upper respiratory tract infection, may cause a relapse, the patient should be instructed to avoid infection whenever possible and to seek prompt treatment of any infections. Any patient with a persistently positive Coombs test result is liable to undergo relapse, even though he may be in complete remission after splenectomy or steroid hormone therapy.

Symptomatic Acquired Hæmolytic Anæmia.—The treatment of symptomatic AHA depends upon the severity of the AHA and the nature of the underlying condition. In less severe cases treatment of the underlying disease may be tried first—for example, irradiation for lymphosarcoma, lymphatic leuchæmia, Hodgkin's disease, or resection for tumours of the ovary or bowel; a remission of the AHA may follow relief of the primary disease. If anæmia is severe, transfusion and steroid

hormone therapy are indicated to maintain life until such treatments are feasible. If treatment of the primary condition is not followed by remission of the AHA, and if that condition itself is not likely to be fatal in the immediate future, management should be as for idiopathic AHA. The response to treatment varies; some patients respond despite persistence of the primary disease, although many do not.

The clinical importance of the recognition of systemic lupus as a cause of AHA lies in the fact that remission of systemic manifestations of the disease frequently follows adequate therapy with steroid hormones, and that prognosis is much improved when diagnosis is made early and treatment instituted before there is irreversible damage to the kidney, brain or heart.

Course and Prognosis

The course of the disease is exceedingly variable. Death may ensue rapidly in severe cases in which there is no response to treatment, but a fatal outcome is much less common since the introduction of steroid hormone therapy. Most patients will obtain at least a partial temporary remission after one or other form of treatment. In chronic cases active hæmolysis may continue for years, the degree of disability being proportionate to the anæmia. One patient (Case 4) has been observed for five years with hæmoglobin values varying between 7·0 and 9·5 grammes *per centum*, with no treatment other than a transfusion of four pints of blood in 1951. She leads an active life and refuses all treatment. Spontaneous remissions are frequent, especially in chronic cases; for this reason the results of treatment are not always easy to assess.

No patient can be said to be permanently cured if the Coombs test result remains positive, even though the hæmoglobin, reticulocyte and serum bilirubin values are normal. The possibility of relapse always remains—the role of infections in causing such relapses has been stressed.

In symptomatic cases the prognosis depends largely on the underlying disease, which in many cases is itself fatal.

Summary

Twenty-two cases of AHA are described; twelve are idiopathic and ten symptomatic. The necessity of a careful search for an underlying disease, especially *lupus erythematosus*, in all cases of AHA is stressed.

Clinical features noted were the greater incidence in females than in males, the frequency of constitutional symptoms and of associated infections, and the absence of jaundice in six patients and of splenomegaly in two.

A positive response to the Coombs test established the auto-immune nature of the hæmolytic process; although the result was invariably positive at some stage of the disease in all cases, it was initially negative in three, and became negative in two after steroid hormone therapy. After splenectomy it usually remained positive irrespective of the clinical result. A "false positive" Wassermann reaction occurred in four of 16 cases tested.

ACTH in adequate doses is now the initial treatment of choice; if no response occurs cortisone should be tried, as some patients respond to one hormone and not to the other. Blood transfusion should be used when necessary to maintain life; but as reactions are not uncommon, in general transfusions should be kept to a minimum, especially if they appear ineffective. Splenectomy is indicated for those patients who do not respond to adequate steroid hormone therapy, and probably for those who respond, but in whom the process remains active and who require large maintenance doses of hormone to control the anæmia.

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RECURRING RENAL DISEASE CAUSED BY AN ENDOGENOUS OVARIAN HORMONE[1]

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THERE is increasing interest in diseases due to tissue damage by substances occurring naturally, or developing as a result of some independent process, within the patient's own organs. Rheumatic fever and acute nephritis are examples, and some clue to the nature of the noxious substance lies in the preceding streptococcal infection. *Polyarteritis nodosa*, diffuse *lupus erythematosus*, scleroderma, dermatomyositis, exfoliative dermatitis, sympathetic ophthalmia, rheumatoid arthritis, acquired hæmolytic anæmia and paroxysmal cold hæmoglobinuria may be due to a similar mechanism, though the antigenic or toxic agent is usually not apparent. Urbach (1942) reviewed the literature dealing with such a concept of disease under the title of "endogenous allergy", and recalled that such a hypothesis, substantiated by animal experiment, was stated by Elschnig in relation to sympathetic ophthalmia as long ago as 1910.

We present the case history of a young woman who suffered recurrent attacks of severe renal and other tissue damage apparently due to an allergic or toxic reaction to the œstrogens produced during her own menstrual cycle.

Urbach (1939) discussed menstruation allergy or toxicosis and reported several cases, and Zondek and Bromberg (1945, 1947) described investigations on 116 women suffering from menstrual symptoms, 73 of whom showed skin reactions to female sex hormones. These authors refer to other case reports in the literature.

Evidence that the conditions were due to allergy depended on the following tests (Zondek and Bromberg, 1947): cutaneous reaction to purified hormones in a suitable solvent; recurrence of such a reaction during a test giving a positive result performed later at another site; recurrence of the reaction during the active stage of succeeding menstrual cycles; passive transfer of the sensitivity to the skin of a normal subject by injection of serum from the patient; positive cutaneous reaction in the patient to serum taken during the active stage of the cycle; relief of symptoms by specific desensitization. However, as all except the last of these depended on the skin's being a reacting organ, negative results could not exclude allergy as the operative mechanism in some cases.

The following reactions have been suggested as due to hormonal allergy: asthma, vasomotor rhinitis, urticaria, acne, angio-neurotic œdema, eczema, migraine, premenstrual tension and fever, *pruritus vulvæ*, conjunctivitis, diarrhœa and keratitis. We have found no reference to renal disease or to any condition which seriously threatened the patient's health.

CASE REPORT

The patient, a female, was born in April, 1931. She suffered from infantile eczema until the age of nine years. She had had measles, pertussis and pneumonia as a child, and an operation for removal of the tonsils and adenoids. She had never suffered from asthma, hay fever or urticaria. Her mother, who was alive and well, had recurrent attacks of hives and had had "rheumatic fever" as a child. The father was alive and well. One brother, three years older than the patient, had recurrent bronchial asthma. The patient had been in good health until April, 1949, when, at the age of eighteen years, she developed the first of a series of attacks which recurred for the next four years. These attacks began with an erythematous rash which soon acquired an annular or serpiginous pattern, the individual lesions having a slightly raised edge and many having a pale centre. It was never associated with pruritus or weeping. The anterior and posterior aspects of the trunk including the neck were usually affected—the upper part of the thighs commonly—and the face, the upper limbs and the lower limbs below the knees only rarely and usually late in the evolution of the eruption. Within a few hours of the onset of the rash, symptoms of toxæmia occurred, with rising temperature, headache, malaise, complete anorexia and, commonly, vague joint pains. At this time the urine became grossly bloodstained. The hæmaturia was painless and there were no other urinary symptoms. There was an associated proteinuria. Within forty-eight hours the rash, temperature and hæmaturia were well established. The temperature was irregular in type, rising to 103° F. Rigors sometimes occurred at this stage.

These three features—erythematous rash, hæmaturia and toxæmia,—were characteristic and constant in all attacks, and although the later ones were not often

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associated with macroscopically evident hæmaturia, there was always a considerable increase in the number of red blood cells seen on microscopic examination. Other features which occurred during some episodes were cardiac palpitations, pain in the loins, red painful areas at the distal ends of the nailbeds, a red, hot, painful swollen joint and, in one attack, acute iritis and episcleritis.

After the initial attack (of April, 1949) the patient had recurrences from August to December, 1949, from August to November, 1950, from January to May, 1951, and throughout the whole of 1952. The concluding stages of the attack of 1950 and those of 1951 and 1952 were observed in the Royal Prince Alfred Hospital, Sydney.

The attacks subsided spontaneously in eight or ten days even without treatment, microscopic hæmaturia and proteinuria being the slowest signs to settle down. The attacks could be terminated within a few hours by the administration of ACTH. Between attacks the patient was free of symptoms, the blood pressure returned to normal, and chemical and microscopic examination of the urine revealed no abnormality. During 1952, however, proteinuria and microscopic hæmaturia became persistent. In addition, the blood pressure rose slowly during the second half of the year and she developed bilateral papillœdema in December, 1952.

During the period of observation in hospital it was noticed that the attacks had a regular periodicity. From January, 1952, onwards (see Figure I) each attack occurred about twenty-one days after the beginning of the previous one, whether it had terminated spontaneously or as a result of ACTH therapy. The patient's menstrual cycle was at that time of about twenty-one days' duration. It was also observed that the attacks commenced at about the middle of the menstrual cycle and lasted eight to ten days (or less if shortened by ACTH therapy), and the menstrual flow commenced at about the time when the attack subsided. The first five cycles of 1952 show this relationship clearly (see Figure I). The next menstrual period was late, and the next attack was severe and prolonged and had not resolved completely when a further attack was due. In the next three months the regularity was lost. This could have been due to the investigatory procedures carried out, which resulted in a number of artificially induced attacks. The regular sequence of attacks recommenced in August.

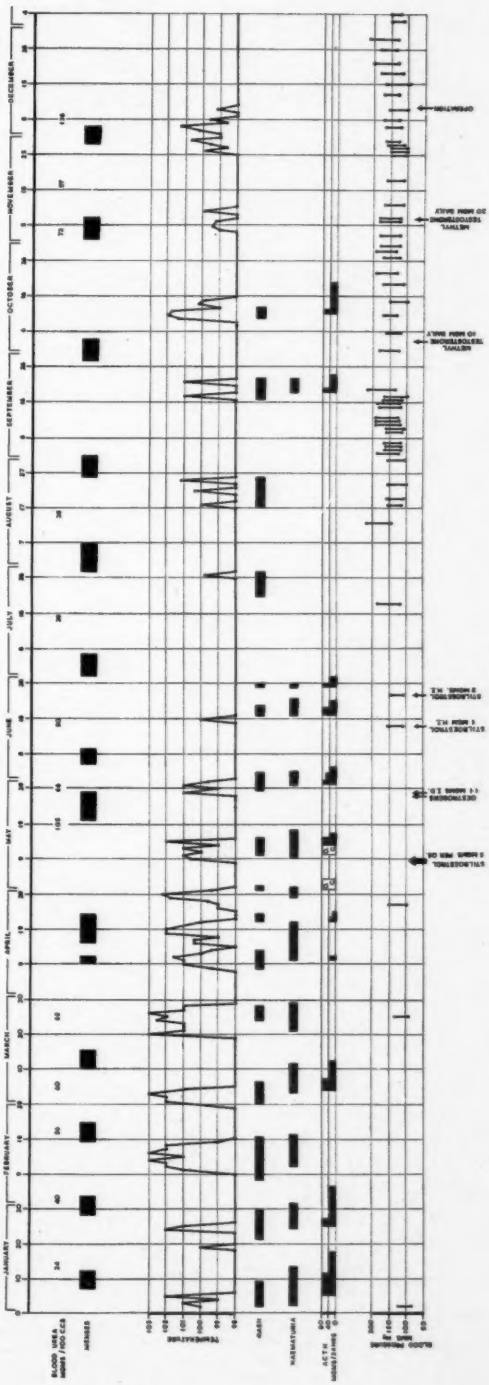

FIGURE I
The course of the illness during 1952.

Investigations

Urological investigation by Mr. M. S. S. Earlam showed the urinary tract to be normal. Bacteriological examination of the urine was performed on 19 occasions; on only three were organisms grown on culture. During 1952 some hyaline casts were usually seen. Proteinuria, which had been a constant finding since the beginning of 1952, varied in amount, increasing during periods of activity. In the latter part of 1952 the urine frequently contained up to one-third protein, as determined by the acidification and boiling test. The specific gravity of the urine became fixed at values between 1010 and 1012. The maximum specific gravity attained on November 4, 1952, after fourteen hours' water deprivation, was 1014.

The blood urea level was elevated during attacks, but usually returned to normal during remissions. For example, an attack commenced on March 2, 1951; seven days later, while symptoms were at their height, the blood urea concentration was 128 milligrammes *per centum*. On March 19 symptoms had subsided,

the patient felt well, and the blood urea level was 51 milligrammes *per centum* and four days later 48 milligrammes *per centum*. On April 10 a further attack occurred and the blood urea level on this day was 40 milligrammes *per centum*. Six days later at the height of the attack it was 90 milligrammes *per centum*. In 1952 this fall in blood urea level did not always occur. The values are shown in Figure I.

The hæmoglobin value dropped slowly during periods of activity and necessitated an occasional blood transfusion. The erythrocyte sedimentation rate was persistently raised to values of 15 to 25 millimetres per hour, estimated by the Hawkesley micro method. The total white cell count was usually increased during attacks to about 15,000 per cubic millimetre, a polymorphonuclear leucocytosis being present. There was no abnormality of blood platelets, bleeding time or coagulation time. The results of many other investigations were negative. These included blood Wassermann and Kline tests, the Widal test, the Mantoux test, the hydatid complement-fixation test, tests for cold agglutinins, attempted blood culture, muscle biopsy, serum protein, plasma cholesterol and plasma bilirubin estimations, Donath-Landsteiner test, red blood cell fragility tests and estimation of urinary porphyrins.

A series of investigations by skin tests, Prausnitz-Kuestner tests and administration of hormones were carried out in an effort to determine the pathogenesis of this disease. The results are shown in Table I and Figure II. Skin tests all gave negative results, but typical attacks followed the oral or subcutaneous administration of œstrogens on four occasions. Two of these may have been fortuitous. In the other two the time relationship made this unlikely.

Treatment

Because of the presence of an organism in an early urinary culture, courses of antibiotics were given, without benefit. It was observed that ACTH had an immediate effect on the course of the attacks. The temperature fell to normal within twelve hours of the

TABLE I

Investigations to Determine Pathogenesis

Nature of Test	Date	Materials	Result	Comment
1. Patch tests.	2/4/52	"Antuitrin S." Œstroform. Progesterone.	No reaction. No reaction. No reaction.	
2. Intradermal tests with hormones.	24/4/52	"Antuitrin S", 0·2 cc. Physiological saline, 0·2 cc.	Erythema (1·7 by 2·8 cm.) at 24 hours. No reaction.	This led to intravenous administration of "Antuitrin S" (see below).
	27/5/52	Stilbœstrol, 0·2 mgm. in ethyl oleate. "Œstroform", 0·2 mgm. in ethyl oleate. Progesterone, 0·5 mgm. in ethyl oleate.	Erythema at 24 hours. Erythema at 24 hours. Erythema at 24 hours.	As a result of later tests, reaction was considered due to ethyl oleate, but there was none available for control tests. Total of 1·1 mgm. of œstrogens.
	28/5/52	Stilbœstrol, 0·3 mgm. in ethyl oleate. "Œstroform", 0·4 mgm. in ethyl oleate.	Erythema at 24 hours. Erythema at 24 hours.	Typical attack occurred on 29/5/52, 20 days after previous one.
	16/6/52	"Œstroform", 0·1 mgm. in watery suspension. "Œstroform", 0·1 mgm. in ethyl oleate.	No reaction in patient or in two controls. Itchy erythema at 24 hours in patient and two controls.	
	3/7/52	Stilbœstrol, 0·1 mgm. in 0·1 ml. arachis oil. 0·1 ml. arachis oil.	Immediate erythema, which faded rapidly. Immediate erythema, which faded rapidly.	
3. "Antuitrin S" given intravenously.	25/4/52 26/4/52	"Antuitrin S", 1 cc. in 9 ccs. saline. "Antuitrin S", 3 ccs. in 7 ccs. saline.	Fever and rigor after 30 mins. Fever and rigor after 30 mins.	There was no rash or hæmaturia, and reaction was considered due to non-specific protein effect.
4. Intradermal tests with serum and follicular fluids.	5/5/52	0·1 cc. of her own serum taken during an attack on 29/4/52.	No reaction.	
	5/5/52	0·1 cc. of follicular fluid taken at operation from a normal luteal cyst.	No reaction.	
5. Prausnitz-Kuestner test on two male and two female normal subjects.	3/6/52	Patient's serum taken during attack.	Stilbœstrol, 0·2 mgm. in ethyl oleate—negative result. "Œstroform", 0·2 mgm. in ethyl oleate—negative result. 0·2 mgm. of "Œstroform" in watery suspension—no reaction.	Erythema and persistent irritation occurred, but this was considered due to the ethyl oleate.
	16/6/52	Patient's serum taken during a remission.		
6. Attacks after administration of œstrogens.	8/5/52	Stilbœstrol, 1 mgm. three times a day by mouth.	Typical attacks after fifth dose (11 days after previous attack).	Given in an attempt to suppress ovulation.
	27/5/52 28/5/52	During a course of intradermal tests recorded above a total of 1·1 mgm. of œstrogens was administered in two days.	Typical attack on 29/5/52 (20 days after previous attack).	Given to investigate skin sensitivity.
	16/6/52	Hypodermic injection of stilbœstrol, 1 mgm., in ethyl oleate.	Typical attack on 17/6/52. (19 days after previous attack).	
	25/6/52	Hypodermic injection of stilbœstrol, 2 mgm., in ethyl oleate.	Typical attack on 26/6/52. (8 days after previous attack).	

first injection, the appetite returned and the rash disappeared; the hæmaturia decreased within the next day or two.

To suppress œstrogen production, treatment with methyl testosterone, 10 milligrammes per day given sublingually, was commenced on October 3, 1952. This delayed but did not suppress either the next menstrual period or the next attack. On November 7, the dose was doubled, but the patient still had a normal menstrual period on November 28 and a further attack.

As has been mentioned earlier, the patient's condition had been deteriorating during the second half of 1952. Persistent albuminuria and a fixed urinary specific gravity were present, with a persistently raised and increasing blood urea content (up to 136 milligrammes *per centum* on December 5), the blood pressure was rising, and early in December papillœdema was noted.

The clinical picture is now that of chronic nephritis with proteinuria, urea retention and hypertension. The hypertension has been treated with hexamethonium bitartrate tablets, 950 milligrammes being given by mouth three times a day. The papillœdema has completely resolved and the visual acuity has returned to normal. Details of this period of observation are recorded in Table II.

DISCUSSION

Relation to Menstrual Hormones

The periodicity of the attacks in phase with the menstrual cycle and their cessation after oophorectomy leave no doubt that an ovarian substance was responsible.

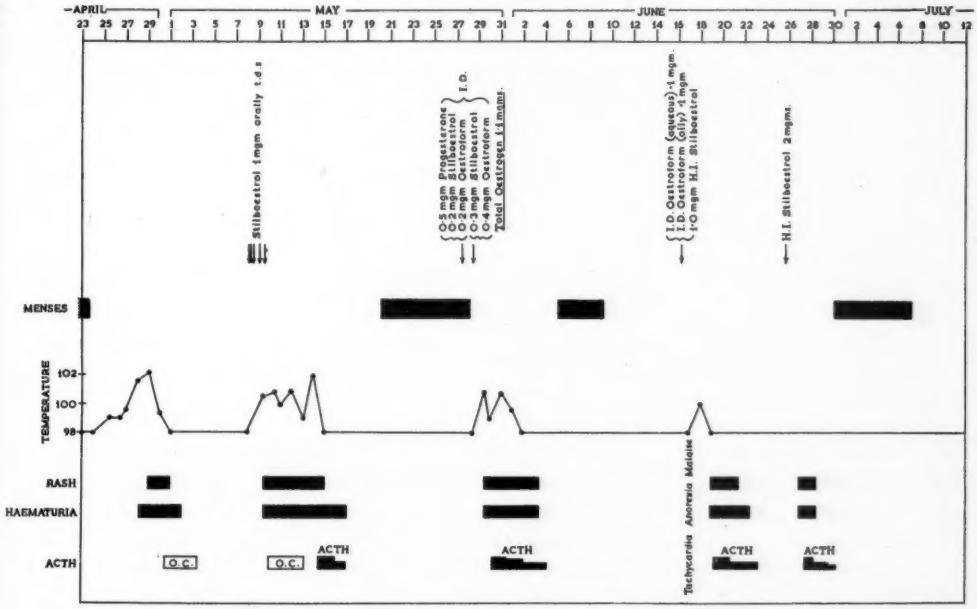

FIGURE II

Five attacks from April to June, 1952. The last four commenced within twenty-four hours of administration of œstrogens. It appeared at the time that those of May 27 and June 18 may have been due to the coincidence of a natural attack, as the regular periodicity had become obscure, but the final attack on June 26, occurring after an interval of eight days, left no doubt of the significance of the œstrogen.

A decision to perform bilateral oophorectomy was then made. On December 10 Mr. Malcolm Stening performed subtotal hysterectomy and bilateral oophorectomy.

Both ovaries were normal in appearance; there was a small fimbrial cyst in the region of one ovary. The kidneys were palpated and were of normal size and contour. Histopathological examination of the ovaries revealed some small follicular cysts and luteal tissue. On the two days after operation a slight rash was present, but otherwise recovery from operation was uneventful.

Follow-Up

There has been no recurrence of the attacks in the nine months since operation, and the patient has felt very well during this period.

The attacks commenced regularly at the mid-menstrual period, and it is probable that the onset coincided with ovulation or followed it closely. Attempts to determine the time of ovulation by daily recording of rectal temperatures were ineffective, for though a rise occurred at the expected time this also coincided with the onset of the patient's febrile attacks. Suggestive evidence that ovulation did occur at this time was provided by the observation of a fall in the number of circulating eosinophile cells twenty-four hours before the onset of an

TABLE II

Data since Bilateral Oophorectomy on December 10, 1952

Date	Blood Pressure. (Millimetres of Mercury)[1]	Blood Urea Content. (Milligrammes *per Centum*)	Albuminuria.	Red Blood Cells in Urine	Remarks
30/1/53	106/105	48	1/4	Occasional red cells in centrifuged deposit.	
10/2/53	193/135	43	1/4	Occasional red cells in centrifuged deposit.	Hæmoglobin value, 12·0 grammes *per centum*.
26/2/53	165/130	48	" Heavy cloud "	Occasional red cells in centrifuged deposit.	
10/3/53	150/110	40	1/4	Occasional red cells in centrifuged deposit.	
31/3/53	140/105	54	1/8	Occasional red cells in centrifuged deposit.	Urea clearance per minute, 41 cubic centimetres (56%).
28/4/53	140/105	50	1/4	Occasional red cells in centrifuged deposit.	
26/5/53	160/120	65	1/10	Occasional red cells in centrifuged deposit.	
30/6/53	140/105	65	" Heavy cloud "	Occasional red cells in centrifuged deposit.	Hæmoglobin value, 11·3 grammes *per centum*.
11/8/53	125/90	61	" Heavy cloud "	Occasional red cells in centrifuged deposit.	
22/9/53	165/120	57	1/10	Occasional red cells in centrifuged deposit.	

[1] Systolic/diastolic.

attack on two occasions (see Figure III). Davis and Hulit (1949) reported that the number of circulating eosinophile cells fell at the time of ovulation in healthy women.

Although the output of sex hormones throughout the menstrual cycle has not been satisfactorily determined, it is the commonly accepted view that œstrogen production rises after the cessation of menstruation, falls at the time of ovulation, then rises again to higher levels up till the onset of the next menstrual period, when the blood level falls abruptly. The duration of our patient's attacks seemed to correspond to the rising concentration of œstrogens between ovulation and the onset of menstruation, and the spontaneous remission of symptoms seemed significantly related to its abrupt fall at the onset of bleeding.

That œstrogens played a significant role in the pathogenesis of symptoms is strongly supported by the onset of attacks within twenty-four hours of the administration of synthetic œstrogens on four separate occasions.

Mechanism

We were unable to obtain direct evidence as to the mechanism of production of symptoms. Many features of the illness suggested that it was due to sensitization of tissues. The hæmaturia suggested acute nephritis or anaphylactoid purpura, the skin rash closely resembled the *erythema marginatum* of rheumatic fever, the joint manifestations and iritis were similar to those occurring in allergic diseases, and the combination of all these in the one patient suggested an allergic mechanism.

The relief of symptoms within four hours of the administration of ACTH was dramatic and invariable. This effect may have been due to modification of the output of the effective hormone, but the rapidity of action is rather against this view, and it is more likely that the ACTH suppressed an antigen-antibody reaction as it appears to do in other forms of anaphylaxis, such as drug allergies.

Attempts to demonstrate skin sensitivity to the female sex hormones were unsuccessful. Follicular fluid taken from a normal luteal cyst during operation was also ineffective.

Prausnitz-Kuestner tests on four subjects with the patient's serum taken during both an attack and a remission, with both oily and watery solutions of œstrogens being used as antigen, were performed with negative results. The patient's serum, taken during an attack, produced no skin reactions in herself during a remission.

It is unlikely that the hormones merely potentiated an unrelated allergic reaction. Hansen-Pruss and Raymond (1943) investigated the antibody titre in women sensitive to ragwort and found that it varied throughout the menstrual cycle, being highest on the last day of menstruation when œstrogens are at their lowest concentration.

Relationship to Glomerulo-nephritis

There were features of the attacks which resembled acute glomerulo-nephritis, including the hæmaturia, proteinuria and cylindruria, hypertension and urea retention. To investigate further this resemblance, serum complement studies were made before and during several attacks. Many investigators have shown that serum complement falls significantly in acute nephritis, and one of us (Reader, 1948) has shown that complement is low during the first few days of the attack and rises rapidly regardless of the subsequent course of the nephritis. We have also found that there is no such fall in complement in patients with anaphylactoid

purpura. The results in this case show that there was no fall in complement throughout the attacks (Figure III). This suggests that the pathogenesis of symptoms resembled that of so-called anaphylactoid purpura rather than that of acute nephritis.

The case also provides information on the mechanism of chronicity in glomerulo-nephritis, suggesting that even though the acute process (for example, the œstrogen effect) ceases, secondary changes are set in operation which become self-perpetuating.

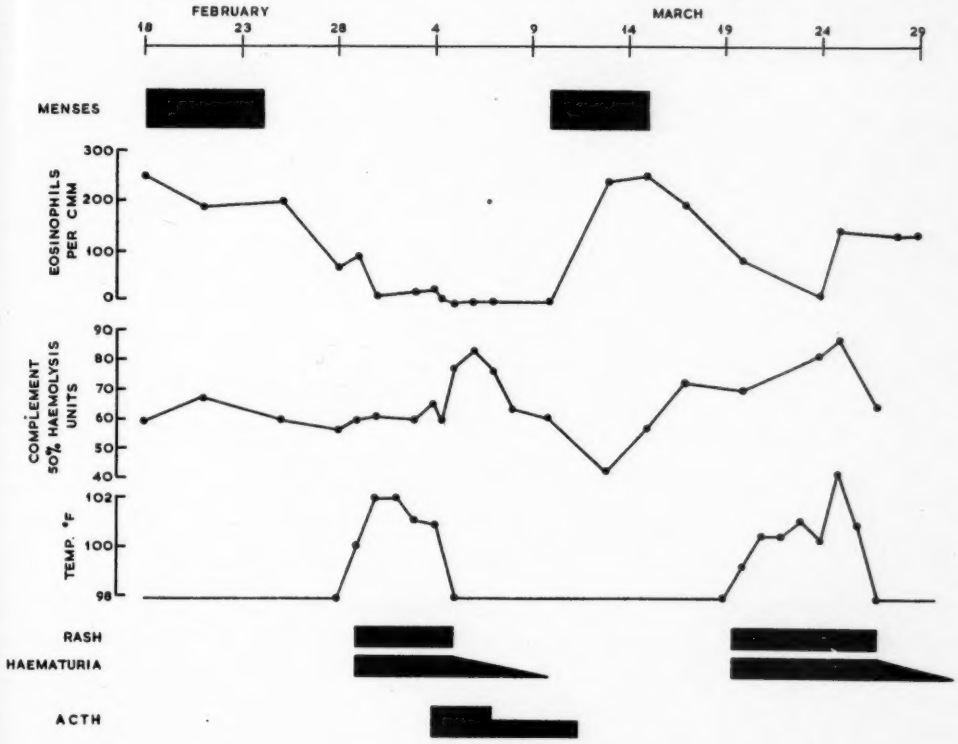

FIGURE III

The eosinophile cell counts and serum complement levels throughout two menstrual cycles. Eosinophile cell counts were made by the eosin and acetone technique of Dunger (1910). Complement is expressed as the reciprocal of the amount of serum producing 50% hæmolysis of a standardized number (125×10^6) of sensitized sheep erythrocytes. Details of the method will be published in a later paper. The rise in complement titre with administration of ACTH has been observed in other cases and will be the subject of a further communication.

Although the patient has had no attack in the eight months since oophorectomy, her renal lesion has persisted, and she now presents the classical picture of chronic glomerulo-nephritis with hypertension and nitrogen retention, and protein, red cells and casts in the urine. The case thus illustrates that the clinical picture of chronic glomerulo-nephritis may follow an acute renal lesion other than acute glomerulo-nephritis. Osler (1914) and Gairdner (1948) have reported chronic nephritis following anaphylactoid purpura, and it is well known that healed acute pyelo-nephritis may have a similar result.

SUMMARY

The case history is presented of a young woman who suffered recurrent attacks of fever with rash, hæmaturia, nitrogen retention and hypertension, for four years.

The attacks commenced at the mid-menstrual period and subsided with the onset of the menses. They could be terminated in a few hours with ACTH. On four occasions attacks occurred within twenty-four hours of the administration of œstrogen. There were many features which suggested that they were due to an allergic mechanism, but we were unable to confirm this by investigation.

The attacks ceased after oophorectomy, but the patient now shows the clinical picture of chronic glomerulo-nephritis.

ACKNOWLEDGEMENTS

Our thanks are due to Professor C. G. Lambie and Dr. A. W. Morrow for their interest and assistance during the investigations, and also to Dr. E. M. Day of the Fairfax Institute of Pathology.

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THE USE OF A CATION EXCHANGE RESIN IN THE MANAGEMENT OF ANURIA IN CHILDHOOD[1]

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FROM July, 1952, to August, 1953, six children with anuria of four to twelve days' duration, were treated at the Royal Children's Hospital, Melbourne. In five the anuria was due to acute glomerulonephritis, and in one to *polyarteritis nodosa*.

The management of all patients was conservative, and followed the general principles of Bull, Joekes and Lowe (1949). The basis of treatment was the replacement of daily water losses, and the diminution of protein catabolism and acidosis by provision of adequate glucose. Diuretics were strictly avoided, and electrolytes were administered only when indicated. Dialysing procedures were not used on any of the six children, but five were treated with a cation exchange resin in either the ammonium or sodium cycle, because of clinical, biochemical or electrocardiographic evidence of potassium intoxication. One child recovered from anuria before the serum potassium concentration reached dangerous levels, and resin was not used. Two of the five children who were treated with resin subsequently died, one six months later from *polyarteritis nodosa*, the other ten days after recovery from anuria, from congestive cardiac failure and unremitting fits.

The clinical use of cation exchange resins in the treatment of anuria has been described by Elkinton *et alii* (1950), by Stock (1952—one patient), and by Bull *et alii* (1953—one patient), and Harthon and Sigroth (1952) treated one patient suffering from hyperkalæmia, the result of terminal chronic nephritis. All these patients were adults, and there has been no description of the use of cation exchange resins in the management of anuria occurring in children.

Analyses of the serum and fæces of three patients demonstrated the efficacy of the resin in withdrawing potassium from alimentary secretions. Fæcal analyses were not made in two cases; but in one of these, whilst the patient was still anuric, a fall in serum potassium level occurred during the administration of resin. The other child excreted urine on the day after the commencement of resin therapy, and assessment of the resin's efficacy in lowering the serum potassium concentration was complicated by renal potassium excretion. However, continued therapy resulted in features of potassium depletion.

Amberlite XE-96[1] in the ammonium cycle was used in treating the first three patients; but for the management of the last two children the resin was converted to the sodium cycle before use. This exchange was made in order to avoid hyponatræmia and acidosis, which developed in the first treated patient. A similar modification has proved successful in the experimental work of Danowski *et alii* (1951) and of Greenman *et alii* (1951), and has been used by Stock (1952) and by Bull *et alii* (1953) in the treatment of anuria.

It is not held that the use of a potassium-free cation exchange resin will correct the disordered physiology of anuria; but there is evidence that one of the established causes of death in anuria—namely, potassium intoxication—can be successfully treated, and by the use of the resin in the sodium cycle increasing acidosis can be avoided. Other possible causes of death during anuria have not been well defined.

CASE REPORTS

CASE I.—The patient was a boy, aged nine years, weighing 38 kilograms, who was admitted to hospital on July 7, 1952, with anaphylactoid purpura, acute nephritis and anuria of one day's duration. The blood urea concentration was 67 milligrammes per 100 mils, and the serum electrolyte concentrations (expressed in milliequivalents per litre) were as follows: sodium 144, potassium 5·0, calcium 4·5, chloride 108, bicarbonate 15·3, phosphate 3·7, sulphate 6·0, protein 11·0.

The child remained anuric for eleven days. Treatment during this period was conservative, and consisted of the administration of at least 50 grammes of glucose daily, and water to replace the loss in sweat, vomitus and stools.

Serum electrolyte estimations were repeated daily, or every second day, and showed progressive changes typical of anuria, so that by the eleventh day of anuria the concentrations (in milliequivalents per litre) were

[1] Received on October 17, 1953.

[1] Amberlite XE-96, Rohm and Haas Company, Philadelphia, Pennsylvania, United States of America.

as follows: sodium 138, potassium 8·3, calcium 4·5, chloride 87, bicarbonate 6·7, phosphate 5·1, sulphate 26, protein 12. The blood urea concentration was 114 milligrammes per 100 millilitres. Auricular extrasystoles occurred, and the electrocardiogram also showed peaked *T* waves, low *P* waves, and widening QRS complexes, which together with clinical and biochemical evidence indicated a state of potassium intoxication. This was treated as an acute emergency with the administration of 30 units of regular insulin subcutaneously and 30 grammes of glucose orally every eight hours. Twelve hours later, a carboxylic cation exchange resin (amberlite XE-95) in the ammonium cycle was administered by stomach tube, in a dosage of 15 grammes every eight hours (45 grammes per day).

To avoid acidosis, hyponatræmia and hypocalcæmia, resulting from the administration of an ammonium cycle resin, 350 millilitres of water with 5% glucose solution, and containing 40 millilitres of 10% sodium bicarbonate solution and 10 millilitres of 10% calcium gluconate solution, were given intravenously as part of the estimated daily water intake.

As a result of this therapy, the serum potassium concentration fell, and the sodium level rose, so that twenty-four hours after commencement of the emergency measures, the serum electrolyte concentrations expressed as milliequivalents per litre were as follows: sodium 144, potassium 6·8, calcium 4·0, chloride 83, bicarbonate 6·2, phosphate 5·2, sulphate 32 and protein 12·4. On the second day of resin therapy, the serum potassium concentration had fallen still further to 6·4 milliequivalents per litre; but the serum sodium concentration had fallen to 124 milliequivalents per litre. The fall in serum sodium concentration was much greater than the fall in potassium concentration despite a much greater potassium content in resinous fæces, suggesting that potassium withdrawal was mostly from the intracellular compartment. Later on the same day the child passed 300 millilitres of urine, and resin therapy was discontinued. Two days later severe diarrhœa commenced; but the concentrations of both sodium and potassium in fæces decreased appreciably, and the sodium concentration exceeded that of potassium. At the same time, serum sodium and bicarbonate concentrations commenced to rise, despite persistence of diarrhœa, and with the reestablishment of urine flow the serum potassium level remained low. Figure I demonstrates these changes. Unfortunately, in this case, the total dried weight of fæces was not measured, and a true assessment of the relative fæcal losses of electrolytes from resinous fæces, and from diarrhœa, could not be made accurately. The serum potassium concentration had begun to fall two days before an adequate urine flow was reestablished, and the high potassium content of non-diarrhœal resinous stools could only be attributed to the resin itself. Despite recovering from anuria, the child later developed hypertension, persistent fits and congestive cardiac failure, and died after two months of coma. Necropsy revealed *polyarteritis nodosa* with terminal bronchopneumonia.

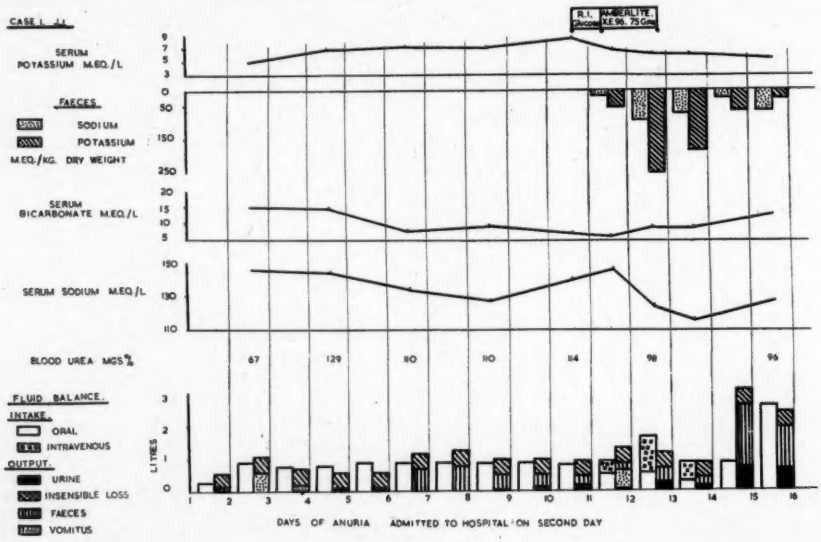

FIGURE I

CASE II.—The patient was a boy, aged seven years, weighing 23 kilograms, who was admitted to hospital on May 6, 1953, with acute nephritis and extreme oliguria present for the previous three days. The blood urea concentration was 320 milligrammes per 100 millilitres, and the serum electrolytes, expressed as milliequivalents per litre, were as follows: sodium 130, potassium 8·2, calcium 5·0, chloride 85, bicarbonate 22·5, phosphate 5·9, sulphate 9, proteins 16·8. The pH of the blood was 7·42. Apart from the bicarbonate concentration, all estimations showed changes typical of anuria.

Treatment consisted of daily replacement of the amount of water lost with glucose and water so that the patient received at least 50 grammes of glucose per day. Although the child was in positive water balance on all but one day of his first week in hospital (Figure II), at the end of this period he was not œdematous. Repeated vomiting made assessment of his fluid intake difficult.

On the day after his admission to hospital, the fifth anuric day, the serum potassium concentration had risen to 9·4 milliequivalents per litre and although no cardiac irregularities were detected clinically, his pulse rate was 40 per minute, and an electrocardiogram showed peaked *T* waves. Potassium intoxication was treated as an acute emergency by the administration of an additional 40 grammes of glucose orally, and 10 units of regular insulin subcutaneously every six hours. Soon afterwards, a carboxylic cation exchange resin (amberlite XE-96) in the ammonium cycle was given orally, in a dose of five grammes every six hours (20 grammes per day), and was continued for six days. Administration of the resin was associated with vomiting on most occasions and replacement was necessary. The total amount of resin retained could not be determined accurately.

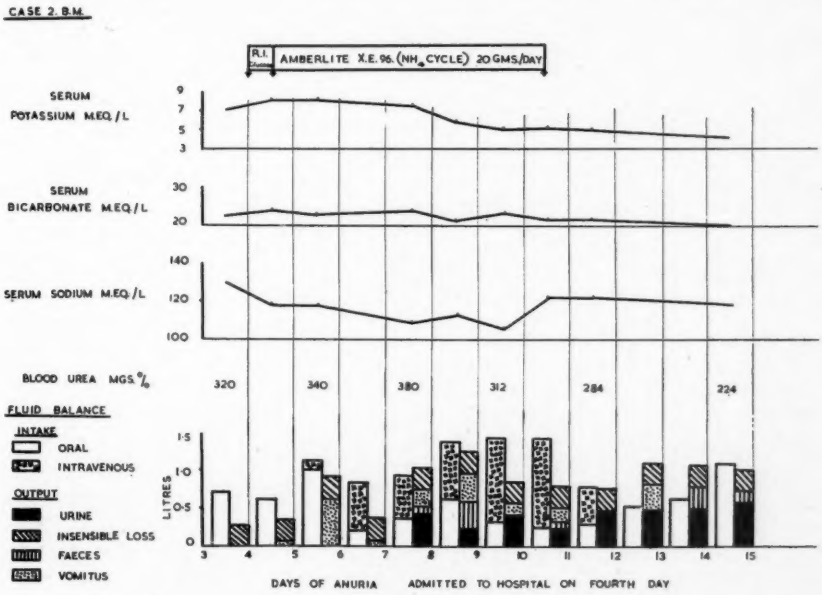

FIGURE II

Twenty-four hours after the commencement of resin therapy, the serum potassium concentration was still 9·4 milliequivalents per litre. The sodium and bicarbonate concentrations were virtually unchanged. Three days after the administration of resin, despite no urine excretion, the serum potassium level had fallen slightly to 8·8 milliequivalents per litre, the bicarbonate concentration was unchanged, but the serum sodium concentration had fallen sharply to 108 milliequivalents per litre. These changes may have been due partly to the dilution effect of water with 5% glucose solution, which had been given intravenously for the preceding two days because of vomiting.

On the eighth anuric day, the fourth day of resin therapy, urine excretion recommenced and remained adequate from then onwards. The serum potassium concentration on the day after the restoration of urine flow showed a sharp decrease to 6·4 milliequivalents per litre, and the administration of resin was discontinued thirty-six hours later, when the serum potassium concentration had fallen to 5·6 milliequivalents per litre. The serum sodium concentration increased, and from the fourth day after the return of urine excretion, the serum sodium, potassium and bicarbonate concentrations remained stable.

This child developed diarrhœa on one day only. Analyses of electrolyte concentrations in fæces were not made, and there is no objective evidence for the resin's part in averting serious ill effects from potassium intoxication. Although his convalescence was slow, the child was discharged from hospital two months after recovery from anuria. He still showed evidence of active nephritis with persistent albuminuria and hæmaturia.

CASE III.—The patient was a boy, aged six years, weighing 19·5 kilograms, who was admitted to hospital with acute nephritis and oliguria of one day's duration. His daily water losses were replaced as water with glucose, so that he received at least 50 grammes of glucose daily. He was still anuric two days after his admission to hospital, and the serum electrolyte concentrations (in milliequivalents per litre) were as follows: sodium 143, potassium 6·4, calcium 4·5, chloride 98, bicarbonate 20, phosphate 4·7, sulphate 1·0, proteins 16. The blood urea concentration was 240 milligrammes per 100 millilitres. Four days after his admission to hospital the child excreted 160 millilitres of urine, but none on the following day, and his serum potassium concentration had risen to 7·5 milliequivalents per litre. There was no clinical evidence of potassium intoxication, but an electrocardiogram showed peaked *T* waves. He was given a carboxylic cation exchange resin (amberlite XE-96) in the ammonium cycle at a dose of five grammes every six hours (20 grammes per day). On the following day, after six days of virtual anuria, he excreted 300 millilitres of urine, but despite the maintenance of a good flow and continued administration of resin, his serum potassium concentration continued to rise, and the bicarbonate concentration fell. After two

days' diuresis, the serum electrolyte estimations in milliequivalents per litre were as follows: sodium 135, potassium 8·1, calcium 4·5, chloride 92, bicarbonate 15·6, phosphate 8·0, sulphate 1·7 proteins 17. The blood urea level had risen to 348 milligrammes per 100 millilitres. The electrocardiogram showed a persistence of peaked *T* waves, but no other changes. For these reasons, although renal excretion had commenced, the same daily dose of resin was continued, but the serum potassium concentration began to fall sharply, so that five days after commencement of the resin therapy, and four days after the restoration of urine excretion, the concentration was 4·2 milliequivalents per litre and the resin was discontinued. Next day the serum electrolyte concentrations in milliequivalents per litre were as follows: sodium 132, potassium 3·5, chloride 86, bicarbonate 23·5. The child had been vomiting for the final four days of resin a mixture containing potassium citrate, for the purpose of promoting diuresis. On the day after his admission to hospital, his blood urea concentration was 95 milligrammes per 100 millilitres, and he was given 500 millilitres of water containing 50% glucose solution in ten minutes, in a further attempt to promote diuresis. When this proved unsuccessful he was transferred to the Royal Children's Hospital, Melbourne, on May 23. At the time of the transfer he was in his fourth anuric day.

The patient was drowsy, with obvious œdema of the face, ankles and sacrum. As it was feared that the administration of potassium night have raised his serum potassium concentration to toxic levels, immediate serum electrolyte estimations were made, and in milliequivalents per litre the results were as follows: sodium 128, potassium 6·2, chloride 75, bicarbonate 20.

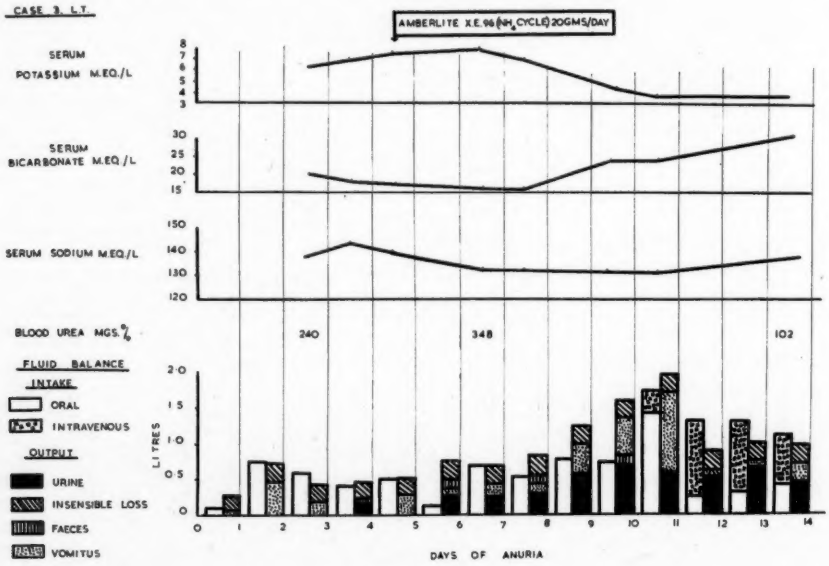

FIGURE III

therapy, and although this resin was replaced, an intravenous infusion of one-quarter isotonic sodium chloride solution with 5% glucose solution was commenced. He was lethargic and hypotonic, and an electrocardiogram showed low *T* waves suggesting potassium depletion. Potassium chloride was added to the infusion fluid, and his clinical condition improved, whilst the electrocardiogram showed a return of *T* waves to normal amplitudes. A low serum potassium concentration persisted and three days later was still 3·6 milliequivalents per litre (Figure III). Analyses of fæces or urine for electrolyte concentrations and total daily excretions were not made.

Subsequently the child recovered without any further complication; but he still had active nephritis with albuminuria and hæmaturia, three months after the onset of his illness.

CASE IV.—The patient was a boy, aged five years, weighing 23 kilograms, who developed acute nephritis with complete anuria and was admitted to a country hospital on May 21, 1953. His treatment included the administration of glucose fluids and orange drinks, and

As the child had been overhydrated, he was kept in negative water balance. Fluid was given as water with glucose so that his daily glucose intake totalled at least 50 grammes. With this regimen his œdema diminished. The administration of a carboxylic cation exchange resin (amberlite XE-96) in the sodium cycle was commenced orally, on the day after his admission to hospital, although there was no clinical or electrocardiographic evidence of potassium intoxication. The dose administered was five grammes every six hours (20 grammes per day). He remained well despite anuria, but two days after commencement of the resin therapy his serum electrolyte concentrations (in milliequivalents per litre) were as follows: sodium 116, potassium 8·4, calcium 4·3, chloride 82, bicarbonate 16·5, phosphate 3·1, proteins 15. The blood urea concentration was 180 milligrammes per 100 millilitres. On the following day, the serum potassium concentration commenced to fall whilst the sodium and bicarbonate concentrations commenced to rise, and these improvements were progressive until, on the eleventh day of anuria, serum electrolyte estimations were made and the results, in milliequivalents per litre,

were as follows: sodium 132, potassium 4·4, calcium 3·8, chloride 85, bicarbonate 21·5, phosphate 6·9, sulphate 7·8 and proteins 16·5. On this day he passed 90 mils of urine. He complained of hunger, so a frugal diet of toast and honey and milk and water was given, as urine was being excreted and the serum potassium concentration was normal. Improvement thenceforwards was progressive and a good urine flow was re-established. Serum electrolyte concentrations became normal, and a light diet was eagerly accepted. Quantitative analyses of resinous fæces demonstrated the potassium-withdrawing activity of the resin, and the results greatly contrasted with the potassium content of his fæces during convalescence (Figure IV). The resin therapy was continued for three days after commencement of urine excretion. One week later he developed a recurrence of generalized œdema and ascites, but this subsided after a week, with spontaneous diuresis. His subsequent convalescence has been uneventful, although he still has active nephritis with hæmaturia and albuminuria.

Serum electrolyte concentrations expressed in milliequivalents per litre on the day when resin therapy was commenced were as follows: sodium 136, potassium 6·2, calcium 6·0, chloride 94, bicarbonate 26, phosphate 4·8, sulphate 3·1, proteins 18·2. The blood pH was 7·44. The child remained anuric for a further six days. On the twelfth anuric day urine flow commenced and became adequate within two days, when the resin was discontinued. Over the period of administration of resin, despite anuria, the serum potassium concentration steadily decreased, whilst serum sodium and bicarbonate concentrations remained fairly stable at slightly subnormal values. Serum electrolyte concentrations in milliequivalents per litre on the day preceding diuresis were as follows: sodium 130, potassium 4·5, calcium 4·5, chloride 80, bicarbonate 21, phosphate 10, sulphate 5, proteins 19·8. The blood urea concentration was 360 milligrammes per 100 mils, and blood pH was 7·43. Analyses of resinous fæces for electrolyte content demonstrated a high potassium uptake by the sodium cycle resin—again,

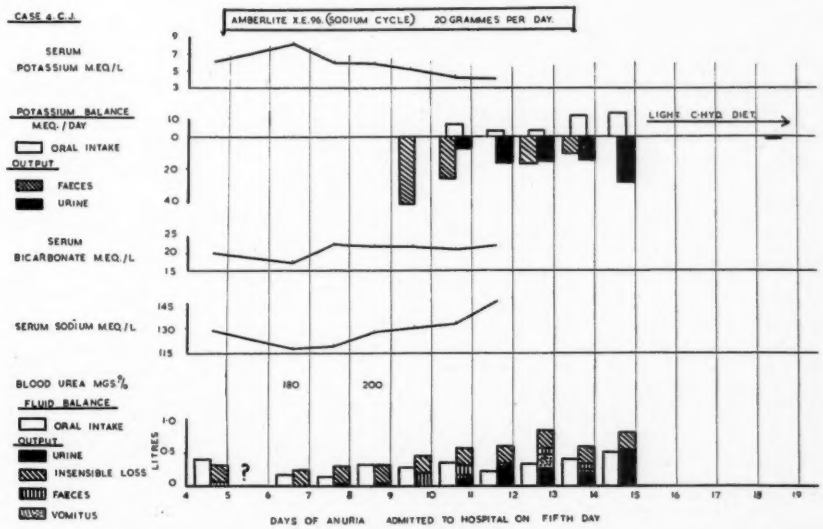

FIGURE IV

CASE V.—The patient was a girl, aged nine years, weighing 27 kilograms, who was admitted to a provincial hospital on June 14, 1953, with acute nephritis, and anuria of one day's duration. She was treated with glucose fluids given by mouth to replace daily losses. Serum electrolyte concentrations on the day after her admission to hospital, in milliequivalents per litre, were as follows: sodium 145, potassium 8·3, chloride 99, bicarbonate 24 and proteins 17. The blood urea concentration was 67 milligrammes per 100 mils. After she had been anuric for five days she was transferred to the Royal Children's Hospital, Melbourne, on June 17. Her daily water losses were replaced as water with glucose, so that she received at least 50 grammes of glucose daily. There was no clinical or electrocardiographic evidence of potassium intoxication, but as the potassium concentration of serum taken two days previously was high, treatment with a carboxylic cation exchange resin (amberlite XE-96) in the sodium cycle was commenced, at a does of five grammes every six hours (20 grammes daily). as in Case IV, greatly contrasting with the potassium content of the fæces before resin was administered (Figure V). On the eighth day of anuria the child had complained of hunger, and as her serum potassium concentration at this stage was 5·9 milliequivalents per litre, she was given a frugal diet of toast and honey, jelly and cream, and a dilute milk and water mixture. This was repeated in two days' time and continued daily. Despite this diet, and the persistence of anuria, the serum potassium concentration continued to fall until on restoration of urine flow it was 4·5 milliequivalents per litre, and later with the excretion of potassium in urine as well as in resinous fæces, it fell to 3·4 milliequivalents per litre.

Over the anuric period she remained fairly well, was drowsy, but did not vomit. At no stage was there clinical, biochemical or electrocardiographic evidence of potassium intoxication.

Two days after her recovery from anuria, her blood pressure was 140 millimetres of mercury, systolic, and 105 millimetres, diastolic, and she commenced to have

convulsions. Despite therapy, fits persisted intermittently for seven days, and finally she became comatose. She was fed by gastric tube, and urine excretion was satisfactory, although the urine was heavily blood-stained. Soon afterwards congestive cardiac failure developed, and despite digitalis therapy, death occurred ten days after her recovery from anuria. Necropsy revealed subacute glomerulonephritis, with obliteration of the capsular spaces of most glomeruli. The lower lobes of both lungs were slightly congested and œdematous.

TECHNICAL PROCEDURES

Serum electrolyte estimations were made by the following methods: sodium and potassium, by the E.E.L. flame spectrophotometer; calcium, by the method of Kramer and Tisdall (1921); chloride, by the method of Schales and Schales (1941); bicarbonate, by the micro-volumetric method of van Slyke (1917); inorganic phosphate, by the method of Fiske and Subbarow (1925); sulphate, by the method of Letonoff and Reinhold (1936); proteins, by the copper sulphate specific gravity method of Phillips *et alii* (1943).[1]

The blood urea content was estimated by urease Nesslerization. The pH estimations were made by means of a Jones pH meter. Venous blood was taken and a micro glass electrode system used, but readings were not made anaerobically. Correction was made for temperature after immersion in a water bath (t° C.) by subtracting (38–t) 0·0147 from the reading (Rosenthal). Fæces were dried and weighed, and aliquots were digested with normal hydrochloric acid for thirty minutes. Sodium and potassium concentrations of the diluted digests were estimated by means of an E.E.L. flame spectrophotometer.

The potassium content of foodstuffs was calculated with the aid of McCance and Widdowson's tables ("The Chemical Composition of Foods", 1940).

DISCUSSION

In each case, the basis of treatment was replacement of the water lost each day from the skin, lungs and stomach. The calculated volume of water with glucose was given orally unless vomiting necessitated an intravenous infusion. On occasions it was difficult to measure the volume of vomitus, so approximations were made. The daily glucose intake was sufficient to achieve maximal protein-sparing effect and minimal acidosis (Gamble, 1947). No attempt was made to provide normal daily caloric requirements by addition of fat (Bull *et alii*, 1949) because of its nauseating effect.

It is possible to remove most metabolic products from the body by means of dialysis, and whilst theoretically this is the method of choice, technical difficulties make it a time-consuming and hazardous procedure, especially

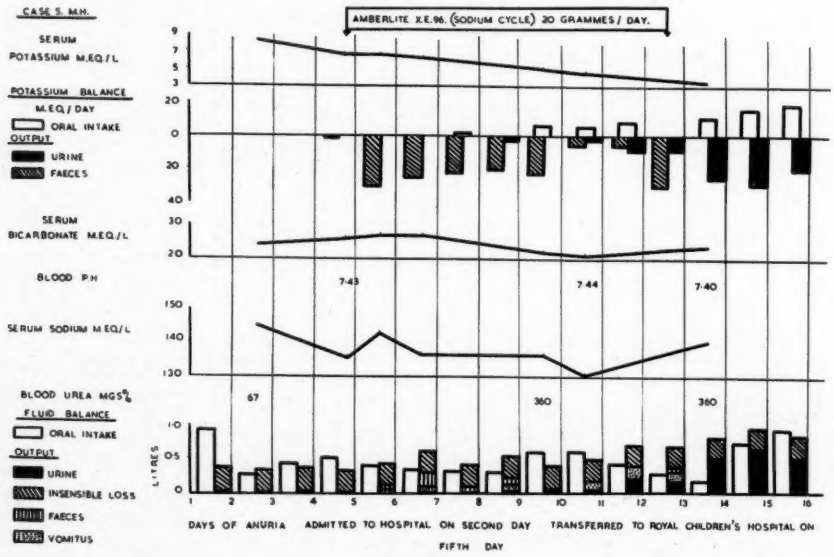

FIGURE V

[1] Phillips, Van Slyke, Dole, Emerson, Hamilton and Archibald, working at the Hospital of the Rockefeller Institute for Medical Research, New York.

in children. There is no evidence that nitrogenous products of metabolism are dangerous, but a well established danger in anuria is potassium intoxication. Five of the six anuric children showed clinical, biochemical or electrocardiographic evidence of potassium intoxication, and a carboxylic cation exchange resin (amberlite XE-96) in the ammonium or sodium cycle was given orally to remove potassium from alimentary secretions. The resin was successful in achieving the desired result, and objective evidence was obtained of its effect in lowering serum potassium concentrations and in extracting potassium in the fæces.

Synthetic resins used in internal medicine for the removal of sodium in œdematous states contain potassium with hydrogen or ammonium in the cation cycle, and are thus useless for the purpose of removing potassium from alimentary secretions. Amberlite XE-96 is not a commercial medical resin, and is coarse, unpalatable and difficult to swallow. However, it is available in the ammonium cycle which is convertible to the sodium cycle, and thus it is suitable for use as a potassium remover. Three children (Cases I, II and III) were treated with the resin in the ammonium cycle, and two children (Cases IV and V) were treated with the resin in the sodium cycle.

In all five cases, the serum potassium concentration fell after treatment with resin had commenced. Two children (Cases I and II) were treated as "acute emergencies" with glucose given orally and regular insulin given subcutaneously, because of clinical and electrocardiographic evidence of potassium intoxication, and soon afterwards resin therapy was started. The fall in serum potassium concentration was maintained in Case I, and initiated in Case II by resin, despite an inadequate urine excretion in both cases. A rapid fall in serum potassium concentration accompanied the restoration of an adequate urine flow in Case II. The serum potassium concentration of one child (Case III) continued to increase after the institution of resin therapy, and after the establishment of urine excretion. Continuation of resin therapy resulted in a low serum potassium level, with clinical and electrocardiographic evidence of potassium depletion. Resins continue to act as exchangers whilst in contact with alimentary secretions, and care should be taken to avoid overtreatment. The serum potassium concentrations of two children (Cases IV and V) fell to normal with resin therapy alone.

Analyses of the stools of three children (Cases I, IV and V) provided good evidence of the potassium-removing effect of the resin. In Case I, the dry weight of the total amount excreted daily was not measured, but the potassium uptake of amberlite XE-96 in the ammonium cycle was more than three times the sodium uptake, expressed as milliequivalents per kilogram of dried stool. Danowski *et alii* (1951) and Greenman *et alii* (1951) have demonstrated that potassium exchanges with ammonium or hydrogen more readily than does sodium. After the resin had been discontinued, the child (Case I) developed severe diarrhœa, but the electrolyte concentration of these stools was much less than that of the formed resinous stool, and sodium was detected in higher concentrations than potassium.

The ammonium cycle was exchanged for sodium in the treatment of two children (Cases IV and V), and after the exchange, the concentrations of sodium and potassium were 5·6 milliequivalents per gramme and 0·002 milliequivalent per gramme of resin respectively. After administration to the patients, potassium was freely taken up by the resin, and in Case IV the potassium concentration of resinous fæces ranged from 1·7 to 2·9 milliequivalents per gramme of dried weight; contrasting with 0·35 milliequivalent per gramme in a subsequent non-resinous specimen. In Case V, the potassium concentration ranged from 1·4 to 2·8 milliequivalents per gramme of dried resinous fæces, contrasting with a concentration of 0·4 milliequivalent per gramme of non-resinous fæces. The sodium concentration of dried resinous fæces ranged from 1·0 to 2·4 milliequivalents per gramme of dried resinous fæces, contrasting with a concentration of 0·2 milliequivalent per gramme of dried non-resinous fæces. Thus, potassium replaced sodium from the sodium cycle resin so that its final concentration was greater than that of sodium per unit of dried weight of fæces. The total excretion of potassium in twenty-four hour specimens of resinous fæces in Cases IV and V was ten to forty times greater than that in non-resinous fæces. These analyses provide good objective evidence of the effective potassium-exchanging activity of the resin in both ammonium and sodium cycles.

The ammonium cycle was replaced by the sodium cycle in the resin to prevent further acidosis and hyponatræmia. On the available evidence it is impossible to state that the resin in its ammonium cycle was responsible for an extension of these changes in Cases I and III, as serum bicarbonate concentrations were low before administration of the resin, and in Case II the bicarbonate concentration remained satisfactory throughout the anuric period. The serum bicarbonate concentrations of the two children (Cases IV and V) treated with the

resin in the sodium cycle remained satisfactory, but in one (Case IV) the serum sodium fell to low levels soon after resin therapy commenced and later returned to normal.

Although there is no evidence that acidosis was avoided by the use of the resin in the sodium cycle, the two children to whom it was given did not become acidotic, and the potassium-extracting effect remained satisfactory.

Two children (Cases IV and V) complained of hunger after approximately one week's treatment with water and glucose. As the serum potassium concentrations had reached safe levels with resin therapy, despite persistence of anuria, both children were given frugal diets of toast, honey, jelly, cream and milk. The serum potassium concentrations did not increase.

All the children vomited whilst anuric, and some after restoration of urine flow. The most severe vomiting developed in the child who did not receive resin. In Cases II and III vomiting seemed to be related to resin administration, but could not be definitely attributed to the therapy.

The excretion of resin was accompanied by mild to moderate diarrhœa in all cases. One child (Case I) developed severe diarrhœa after therapy had been discontinued and after urine excretion had been reestablished.

SUMMARY

Five children with anuria and with clinical, biochemical or electrocardiographic evidence of potassium intoxication were treated with a carboxylic cation exchange resin (amberlite XE-96). Three children were treated with the resin in the ammonium cycle, and two with the resin in the sodium cycle.

The serum potassium concentrations of four children returned to normal levels. One child developed clinical, biochemical and electrocardiographic evidence of potassium depletion.

Analyses of the fæces of three children provided good evidence that the resin was an effective potassium extractor.

All five children recovered from anuria. One child died six months lafter from *polyarteritis nodosa*, and one child died ten days after recovery from anuria from further complications of acute nephritis.

ACKNOWLEDGEMENTS

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EXPERIMENTAL OBSERVATIONS IN CHRONIC NEUTROPENIA[1]

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WE record here the case history of a patient who has had neutropenia of unknown cause for eleven years and yet has not suffered from repeated infections. We describe also the effects produced by exercise, adrenaline, cortisone, infection, and the mixing of the patient's plasma with white cells from a normal person.

The normal range of the total leucocyte count is 4000 to 11,000 cells per cubic millimetre of blood, and of neutrophile cells 1500 to 7500 per cubic millimetre (Osgood *et alii*, 1939). Counts which fall below the lower limits of normal constitute leucopenia and neutropenia, conditions which occur commonly together in an acute, chronic or recurrent form. The majority of cases of chronic leucopenia are associated with diseases of bone marrow (such as aplastic anæmia), diseases which have produced splenomegaly (hepatic cirrhosis, portal thrombosis and Felty's syndrome), and liver factor deficiency (as in steatorrhœa and pernicious anæmia) and long-standing anæmia due to iron deficiency or hæmolysis. A familial occurrence has been reported in a few instances (Cesar, 1943; Bousser and Neydé, 1947). The remaining cases are examples of idiopathic chronic neutropenia, in some of which the condition has been curiously cyclical (Reimann and de Berardinis, 1949), in others persistent (Adams and Witts, 1949; Spaet and Dameshek, 1952). However, whether the neutropenia is acute or chronic, idiopathic or associated with other disorders, a prominent feature of the condition is increased susceptibility to infection. It is in this regard that our patient is so unusual.

CASE HISTORY

E.G. (Sydney Hospital Records 502–791, Number 6) was born in 1902 in London of Jewish parents. At the age of two years she went to Singapore, where she stayed till 1941. As a child she had measles, rheumatic fever at the age of ten years, an appendicectomy at sixteen years and diphtheria at eighteen years. A laparotomy was performed when she was two months pregnant because she was thought to have fibroid tumours. She married in 1922, and had one child by Cæsarean section in 1923. Her husband died of tuberculosis in the same year, but the patient has never had any signs of that disease. One year after the birth of her child she underwent an operation for repair of an incisional hernia. She worked in Singapore as an untrained nurse. In 1938 curettage was performed because of severe vaginal bleeding. Menstruation had previously been normal. In 1942 she attended a hospital in Sydney with menorrhagia and vaginal discharge of three months' duration. Her uterus was curetted and she was treated with the intrauterine application of radium. Later in the same year she was readmitted to hospital with recurrence of vaginal bleeding and discharge, swollen knees and ankles and loss of weight. An ulcer was found on the cervix and a total hysterectomy was performed, one ovary being left. Histological examination showed the ulcer to be innocent. Twelve days later she developed a fever and hot, painful joints—fingers, wrists, elbows, ankles and knees. She also complained that her hair was falling out. A diagnosis of multiple infective arthritis was made, and it was suggested that she might also have hypothyroidism.

It was during this period in hospital in 1942 that neutropenia was first noted (see Table I), but no particular attention was paid to it. The total white cell count was 2500 per cubic millimetre, 50% being neutrophile cells. Treatment included the administration of thyroid extract, immobilization of joints and a transfusion of blood which caused a severe febrile reaction. The Wassermann test produced a doubtfully positive reaction, the Kline test produced a positive reaction, and she was given four injections in two weeks of quinine iodobismuthate in arachis oil. The serological tests then gave negative results. A second blood transfusion caused another sharp reaction. She improved steadily and was discharged after three months in hospital to continue physiotherapy as an out-patient. For nine of the twelve weeks in hospital she was febrile, her temperature ranging from 99° to 101° F. She soon regained her normal health and remained well for four years.

In 1946 she was again admitted to the same hospital. Six days previously she had developed burning of the eyes, lachrymation, photophobia, shivering and sweating. Lumps then appeared on the ankles and spread about the knees, wrists, forearms, arms, face and chest. She complained of burning and tingling in these lumps, which were tender, indurated and in some places confluent. In addition the forearms and legs were diffusely swollen and reddish-blue in colour. Two days after the rash began she was given an injection of anti-tetanus serum and a short course of 20 grammes of sulphonamide for a superficial injury. On her admission to hospital the only other findings were coarse rales in the lungs, which, however, were radiologically clear. A diagnosis of *erythema nodosum* was made. No blood counts were made and recovery was uneventful.

In 1948 she was readmitted to the same hospital with symptoms of progressive weakness and giddiness.

[1] Received on December 7, 1953.

[2] Senior Clinical Research Fellow, Kanematsu Memorial Institute of Pathology, Sydney Hospital.

TABLE I

Peripheral Blood and Sternal Marrow Counts, 1942 to 1953

Date	Peripheral Blood (Cells per Cubic Millimetre)		Sternal Marrow			
			Leucocytes	Granulocytes as Percentage of All Cells		
	Total Leucocytes	Neutrophile Cells		August, 1948	March, 1951	November, 1952
October, 1942	2500	1250	Myeloblasts	0·9	1·6	0·6
November, 1942	3800	—	Premyelocytes	2·6	10·8	2·4
July, 1948	3000	660	Myelocytes:			
August, 1948	4000	1040	Neutrophile cells ..	12·0	25·8	16·2
October, 1950	3100	560	Eosinophile cells ..	2·6	2·0	1·2
February, 1951..	2750	350	Basophile cells ..	—	—	—
June, 1951	3800	530	Metamyelocytes.. ..	15·5	22·6	21·2
October, 1951	2800	390	Polymorphonuclear cells:			
March, 1952	3400	340	Neutrophile cells ..	33·5	11·2	29·4
September, 1952	3100	460	Eosinophile cells ..	1·7	0·4	1·0
January, 1953	2900	870	Basophile cells ..	—	—	—
July, 1953	2600	310				
October, 1953	2900	640				

No abnormalities other than ulcers on both tonsils were found. A blood count revealed severe neutropenia. The results of blood and marrow examinations are shown in Table I. Treatment included blood transfusion, and the administration of "Pentnucleotide", liver, folic acid and pyridoxin. There were no signs of endocrinal abnormality, the chest, sinuses and long bones were radiologically normal, and the centrifuged deposit of urine was normal. It was suggested that the patient's unusual diet might be partly responsible for her neutropenia. All her life she has consumed large quantities of raw fruit and vegetables, including onions every day but with adequate amounts of protein as fish and poultry. The final diagnosis was agranulocytosis of unknown ætiology.

The patient first attended the Hæmatology Clinic of Sydney Hospital in 1951 complaining of weakness and vague ill health. She did not suffer from any infections and only rarely did she catch a cold, which invariably was mild and of short duration. No abnormalities other than neutropenia were noted, and exhaustive questioning did not reveal the cause. The result of a fractional test meal was normal, and the results of the Wassermann and Kahn tests were negative. There was no change in her condition during the next year, and she was admitted to hospital for investigation in November, 1952.

Examination showed the patient to be a small, active, voluble female, whose behaviour and quick speech contrasted with her constant complaint of lethargy. Her weight was 55 kilograms and her height 1·47 metres. The skin was thin, shiny, hairless, sallow, finely wrinkled and slightly pigmented in the exposed parts. It resembled the atrophic skin of hypopituitarism. The hair of the head was normal, the eyebrows were very thin, axillary hair was absent and pubic hair was scanty. Comparison with photographs taken earlier in her life showed a great diminution of her eyebrows, but no other pronounced changes. An abdominal incisional hernia was present. The liver and spleen were not palpable. The blood pressure was 140 millimetres of mercury, systolic, and 90 millimetres, diastolic.

Blood examination gave the following results: the hæmoglobin value was 13 grammes per 100 millilitres, the erythrocytes numbered 4,500,000 per cubic millimetre, 200,000 platelets being present, and the leucocytes numbered 2,400 per cubic millimetre, 8% being neutrophile cells, 83% lymphocytes, 7% monocytes and 2% eosinophile cells. The mean corpuscular volume was 92 cubic μ, and the mean corpuscular hæmoglobin concentration was 32%. Biochemical investigations gave normal results for serum sodium, potassium, chloride, urea nitrogen and creatinine contents. The 17-ketosteroid excretion was 8·3 milligrammes in twenty-four hours, the serum cholesterol content was 235 milligrammes per 100 millilitres (ester 172 milligrammes), and basal metabolic rate was —3%. The result of the Kepler test was negative. A glucose tolerance test gave a high normal result. An insulin tolerance test (six units) gave normal results.

Representative results of the white cell counts and marrow examinations made over a period of ten years are shown in Table I. Persistent leucopenia and neutropenia have been present, not cyclical, and without any disturbance of platelets and erythroid elements. Smears of aspirated sternal marrow have appeared normally cellular with normal relative numbers of granulopoietic elements and, apart from the suggestion of arrest at the metamyelocyte level in 1951, normal maturation.

EXPERIMENTAL OBSERVATIONS

Effect of Exercise

The response to exercise was judged by the changes in total and differential counts made immediately before, immediately after and then fifteen, thirty and sixty minutes after exercise. The exercise was taken fasting and consisted of walking briskly up and down two flights of steps (20 steps) eight times in five minutes. Figure I shows that there was an immediate increase of over 50% in the total number of white cells which was mainly composed of neutrophile cells. The neutrophile cells were examined for the various stages of maturity and the relative proportions plotted after the style of the Arneth-Cooke count (Whitby and Britton, 1946). It is evident that no shift in the distribution occurs (Figure II). This rapid and temporary increase in

neutrophile cells without alteration in maturity is the normal response to exercise (Garry and Bryan, 1935). It is thought to be due to the accelerated circulation sweeping out cells which have become sequestrated in various organs of the body.

Effect of Adrenaline

A subcutaneous injection of 0·5 milligramme of adrenaline hydrochloride resulted in the changes shown in Figure III. There is a monophasic rise in leucocytes composed mainly of lymphocytes with a much smaller increase in neutrophile cells. The peak of neutrophile cells occurred at thirty minutes and then gradually returned to normal in four hours. The number of eosinophile cells dropped from 69 to 39 per cubic millimetre at four hours. Initially the increased number of neutrophile cells was composed of the more mature forms,

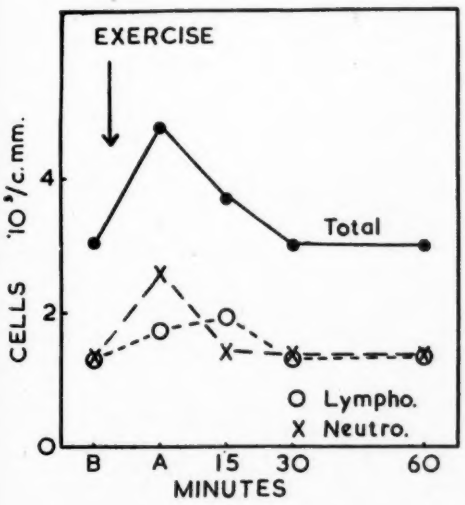

FIGURE I

Cell counts before (B), immediately after (A) and at intervals after exercise

two and three lobed cells, but an increased number of young forms appeared in the blood at two and four hours (Figure IV). This shift to the left appeared to be quite definite (by statistical analysis $\chi^2=43\cdot92$, $n=15$, $p<0\cdot001$), but we have not had the opportunity of repeating the experiment.

The response of normal subjects to adrenaline is usually stated to be a diphasic one. The first peak occurs about thirty minutes after the injection and is due to a great increase in lymphocytes. The second peak occurs about three hours after the injection and the increase is made up almost entirely of neutrophile cells. The neutrophile cells sometimes show a small early rise and fall, but they reach their maximum in the second peak. Chatterjea, Dameshek and Stefanini (1953) have extensively investigated the effects of adrenaline and found no alteration in the maturity of the neutrophile cells. They attribute the increase in circulating white cells to release from various organs,

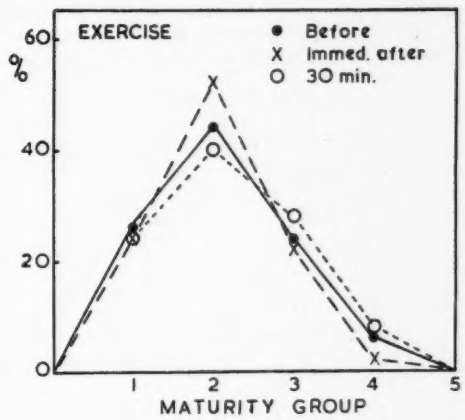

FIGURE II

The maturity distribution of neutrophile cells in relation to exercise. Grouping is based on lobulation of the neutrophile nucleus (Arneth-Cooke)

perhaps including the bone marrow, but with no actual stimulation of marrow tissue, and state that the mechanism probably involves pituitary-adrenal stimulation. The rise in neutrophile cells is said to be less than normal in patients with Addison's disease (Gabrilove *et alii*, 1949). Certainly the spleen is not essential for the response, and the extent of the response to adrenaline is of no diagnostic value except that a poor response is produced in patients with aplastic or hypoplastic marrow. There is a wide range of response in normal subjects.

The response of our patient is in no way abnormal except for the apparent increase in immature cells. The rise in neutrophile cells does not look impressive, but represents in fact 70% of the initial count.

Effect of Cortisone

The patient was given cortisone acetate by mouth, 300 milligrammes the first day, 150 milligrammes the second day, and then 100 milligrammes daily in two doses for four days. Total white cell counts were made in duplicate, and slides were prepared for differential counting

each morning and afternoon, from two days before the commencement of cortisone therapy until seven days after administration of the drug had stopped. Absolute counts of eosinophile cells were made each morning. Aspiration marrow biopsy was performed two days before cortisone therapy commenced, on the third day of treatment and again two days after cortisone therapy had been stopped.

The changes induced by cortisone in the numbers of circulating white cells are shown in Figure V and are similar to the response to the drug shown by other subjects (Finch *et alii*,

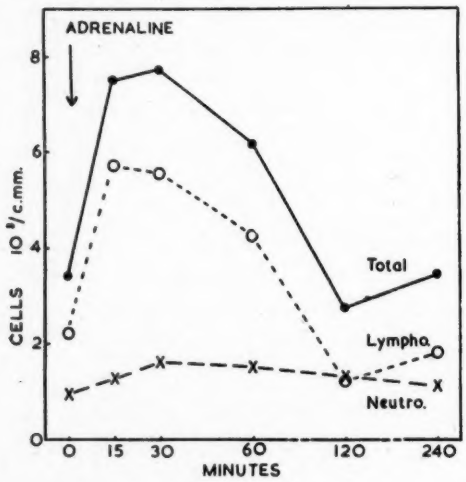

FIGURE III

The effect of adrenaline on circulating white cells

1951). Eosinopenia, an increase in the number of neutrophile cells and a temporary lymphopenia were produced. The total white cell count rose from an initial level under 3000 per cubic millimetre to about 4500. The neutrophile cells increased from 500 to a maximum of 2200 per cubic millimetre on the morning of the second day, and then remained at about 1700 per cubic millimetre throughout the course. When cortisone therapy stopped there was a sharp rebound increase in eosinophile cells and lymphocytes and then a gradual return of the lymphocyte and neutrophile cell counts towards their original values. Even after seven days the neutrophile cell count was still double the pre-cortisone level.

Cortisone produced a "shift to the left" in the neutrophile cells. There was a significant increase in the proportion of immature cells throughout the course of treatment and then a rapid return to the original maturity distribution as soon as treatment was stopped. Representative counts showing the "shift to the left" at the commencement of cortisone therapy and the "shift to the right" at its termination

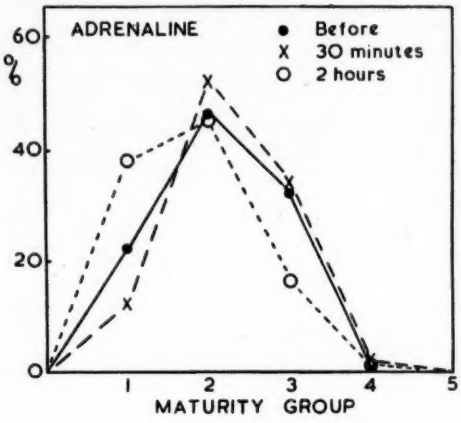

FIGURE IV

The effect of adrenaline on maturity distribution of neutrophile cells

are given in Figure VI. This suggestion that cortisone stimulated the marrow to produce more neutrophile cells or perhaps to release more of them is supported by the findings in

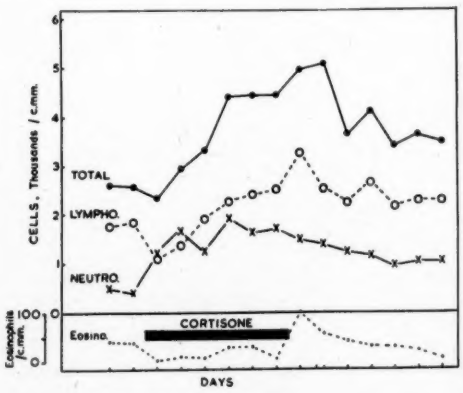

FIGURE V

The effect of cortisone on the mean daily cell counts

the sternal marrow. The differential counts showed a "shift to the left" in the granulopoietic cells which is illustrated in Figure VII.

Our patient responded in a normal fashion to cortisone in so far as the changes in the

peripheral blood picture are concerned. However, it is not so certain that the "shift to the left" among the circulating neutrophile cells and the changes observed in the aspirated marrow tissue are normal effects of cortisone, as we have been unable to find any published evidence on these points. It is quite likely that they are normal, and that cortisone stimulates granulopoiesis just as it sometimes accelerates erythropoiesis (Finch *et alii*, 1951). The assumption that this is so has led to cortisone being used in the treatment of acute

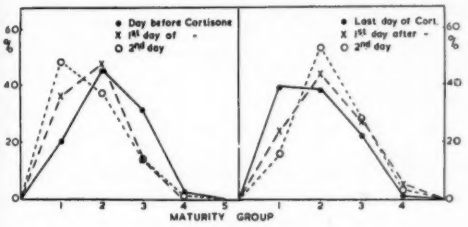

FIGURE VI

Changes in the maturity distribution of circulating neutrophile cells caused by cortisone

agranulocytosis (Caldwell *et alii*, 1950). Spaet, Rosenthal and Dameshek (1951) reported an increase in the white cell count and evidence of increased granulopoiesis in the marrow in two cases of aplastic or adynamic anæmia treated with cortisone or ACTH, and in one other case of typical aplastic anæmia a complete remission was obtained. In one case of chronic neutropenia with hypoplastic marrow whole extracts of adrenal cortex produced no response (Spaet and Dameshek, 1952).

Effect of Plasma on Normal Cells

It is conceivable that, in some patients who have leucopenia in spite of an apparent adequacy of white cell production in the marrow, there is a factor present in plasma which destroys white cells. The possibility of this occurring in our patient was tested on three occasions in the following manner. Heparinized blood from the patient and a normal control subject having the same blood group was centrifuged in graduated tubes, the plasma was removed and then added to the packed cells so as to give a uniform hæmatocrit, and the following four combinations: normal cells with normal plasma, patient's cells with patient's plasma, normal cells with patient's plasma and patient's cells with normal plasma. Duplicate white cell counts were made from each of these four tubes after gentle but thorough mixing, and the tubes were then placed in a water bath at 37° C. The counts were repeated one and three hours later. Smears were made at the same time for differential counts. A random order was followed for sampling and counting. This experiment was carried out before, during and after the course of cortisone, a different control subject being used each time. The results were treated by the analysis of variance and gave similar conclusions on each occasion.

There was no evidence of destruction of normal white cells by the patient's plasma ($p > 0{\cdot}2$). The white cell count decreased during stagnation, but cells in homologous plasma were affected to the same extent as cells in heterologous plasma. The fact that neutrophile cells adhere to glass which is not coated with silicone probably accounted for these

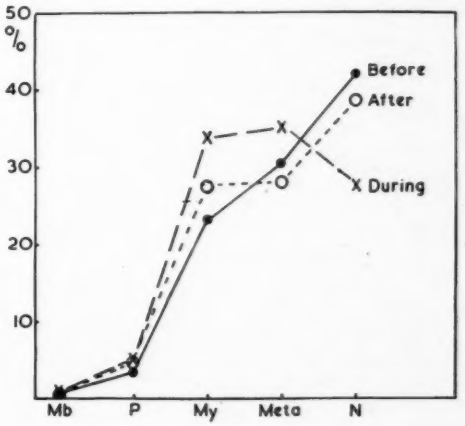

FIGURE VII

The effect of cortisone cn maturity of granulocytes in the sternal marrow. Mb, myeloblasts; P, premyelocytes; My, myelocytes; Meta, metamyelocytes; N, neutrophile polymorphonuclear cells

changes (Hanks and Kingsland, 1949). Doan (1926) has shown that the plasma of some patients destroys the neutrophile cells of other patients, and suggested that white cells have grouping characteristics similar, but not related, to those of red cells. If white cell incompatibility of this normal type had occurred in our cross-matching experiments a false conclusion might have been reached. This criticism can be made of the report by Rutledge, Hansen-Prüss and Thayer (1930), who found in one experiment that the serum of a patient with cyclic neutropenia caused dissolution of the cells of a normal subject and also impaired their motility and phagocytic ability. Roberts and

Kracke (1930) mixed the blood of a patient suffering from relapsing agranulocytosis and a normal subject, but found no evidence of destruction of cells by plasma. In their case, however, the marrow was hypoplastic, which could account for neutropenia without the need for postulating peripheral destruction.

We have not seen any evidence that plasma ever destroys white cells in the same patient; but a process like this, analogous to the destruction of red cells in acquired hæmolytic anæmias, could explain the occurrence of neutropenia in some patients in whom granulopoiesis appears adequate and who are not suffering from hypersplenism. It would be interesting to estimate the lifespan of neutrophile cells in these patients.

Effect of Infection

One year after these experimental observations were made the patient was readmitted to hospital with infection of her leg following an injury. She had been febrile and had been treated with penicillin for three days before her admission. At hospital she had no fever, and her white cell count was 2900 per cubic millimetre, 22% being neutrophile cells. There was extensive cellulitis of the lower half of her leg and a purulent discharge from the abrasion. No organisms were isolated. At no stage during her rapid recovery under treatment with penicillin did the white cell count increase. The hæmoglobin value was 13·8 grammes per 100 millilitres, and there were 213,000 platelets per cubic millimetre.

For the purpose of testing whether cortisone would still produce an increase in the neutrophile cells she was later given five doses each of 100 milligrammes of cortisone acetate by mouth over a period of thirty-six hours. The cortisone appeared to be as effective as previously, the level of neutrophile cells rising to more than 2000 per cubic millimetre.

Discussion

The cause of neutropenia in this patient is not known. The only suspicious features in her history prior to the time when it was first noticed are radium (which was applied to the *cervix uteri*) and her unusual, though apparently adequate, diet. The illness during which neutropenia was first observed had several features in common with disseminated *lupus erythematosus* and an endocrinal disturbance was suspected; but the subsequent course and investigations have failed to confirm these or to offer any more definite cause of the leucopenia. The blood disorder is confined to leucocytes, and the leucopenia has been constant and non-cyclical and has persisted in spite of an apparent adequacy of white cell production in the sternal marrow. But there is no direct evidence of an increased rate of destruction of white cells. The spleen is not palpably enlarged, the tests *in vitro* gave no indication that the plasma is inimical to normal cells, and the slow rate of decrease of the white cell count after cortisone therapy had been stopped is suggestive of a normal survival time of the neutrophile cells once they have entered the circulation.

The most remarkable feature in this case is the absence of infection. The patient has never been troubled unduly by infections, and she seems always to have overcome minor infections in a normal manner. In fact, apart from her complaint of lethargy, which could be explained without the invoking of an organic lesion, there is no reason for suspecting that she is anything but normal. We thought that any threat of bacterial invasion probably would provoke a normal response of white cells, as does exercise, adrenaline and cortisone. It was surprising, therefore, to find the neutropenia persisting in the presence of a severe infection. An interesting observation in our case is the contrast between the apparent unresponsiveness of the neutrophile cells to infection and the facility with which the neutrophile cell count is increased by cortisone. The effect of cortisone contrasts with the ineffectiveness of the stress of infection. Perhaps infection fails to stimulate production of the leucotactic substance normally responsible for causing leucocytosis, or perhaps the marrow is at fault and fails to respond to the stimulus. Our observations allow of no definite conclusions.

Apart from the recent infection—and even this may not have been due to the leucopenia—there is no reason to suspect that the function of the neutrophile cells, quite apart from their absolute number, is at all abnormal. Hickie (1948) showed that the neutrophile cells of a patient with chronic agranulocytosis who suffered repeated infections had subnormal bactericidal powers and that the serum inhibited the activity of cells from normal persons. Rutledge, Hansen-Prüss and Thayer (1930) demonstrated similar inhibitory effects in the serum of a patient who had had cyclic neutropenia and recurrent infections all the nineteen years of her life; but only one experiment was carried out, and the possibility of a normal reaction to white cell incompatibility is not excluded.

It is unusual for patients with neutropenia not to be troubled by repeated infections. In acute cases they commonly present with agranulocytic angina, and in all the reported cases of chronic neutropenia so far as we are aware the patients have had infections, except one patient mentioned by Lawrence (1946).

This was a female who had persistent leucopenia for sixteen years and thrombocytopenia for seven years without infections. The blood count became normal after splenectomy. It is not recorded whether the spleen was enlarged. Splenectomy would have to be considered for our patient if she began to have repeated infections or if other blood elements were affected, particularly if the marrow became hypercellular and the spleen palpable, to complete the picture of splenic neutropenia as described by Wiseman and Doan (1939). Splenectomy could then reasonably be expected to relieve the condition. When any of these features is lacking, the results of operation have usually been disappointing (Adams and Witts, 1949; Spaet and Dameshek, 1952).

SUMMARY

The case history is given of a patient who has had neutropenia for eleven years and yet has not been troubled by infections. The cause is not known; the sternal marrow appears normal and the spleen is not palpable. Other blood cells are not affected.

The white cell count increased normally in response to exercise, and to the administration of adrenaline and cortisone. The patient's plasma did not *in vitro* destroy the white cells of normal subjects. The white cell count did not increase during infection.

ACKNOWLEDGEMENTS

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A STUDY OF NODULAR GOITRE USING RADIO-AUTOGRAPHY AS THE MAIN METHOD OF EXAMINATION[1]

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DIFFICULTIES experienced in the diagnosis and pathological classification of nodular goitre are partly due to the limitations of orthodox methods of examination, particularly with regard to the correlation of functional changes.

Changes in body metabolism have been used as a clinical index of thyroid function, but the information is indirect and is applicable only by inference to tissue changes within the gland. On the other hand, the use of radio-active iodine in clinical work has made it possible, by means of the Geiger-Müller counter, to assess changes of function in portions of the gland itself and even in individual nodules ; but only the average activity of a certain volume of tissue, in which there may be great variation in the microscopic appearance and functional state, is recorded. The deficiencies of localization inherent in these methods are largely overcome by radio-autography, by which the microscopic details of the tissue may be directly related to its radio-active iodine content, the greatest degree of resolution of all the methods available being thus provided.

This study is based upon the individual scrutiny of radio-autographs from 19 cases of nodular goitre in humans including 11 cases of carcinoma, and from 30 carcinoma transplants in rats.

Many isolated radio-autographic observations have been made in thyroid disease ; but these appear to have been interpreted largely in terms of preexisting classifications and have added little to existing knowledge of the pathology and classification of thyroid disease.

MATERIAL AND METHODS

The material was obtained from patients referred for investigation to the Radio-active Iodine Panel at the Royal Melbourne Hospital.[3]

Tracer doses of I^{131} were administered orally to the patients about fifteen hours prior to thyroidectomy or biopsy. The patients in whom carcinoma of the thyroid was known to be present received 200 or 300 μc, whilst the remaining patients were given doses of 100 μc of I^{131}.

The tissue for microscopic examination of sections was selected when possible to include the neighbouring unaffected thyroid gland, so that a comparison between the unaffected and nodular tissue could be made in the same radio-autograph. It was " fixed " in formol-saline and embedded in paraffin. The sections, cut on a rotary microtome set at 9·0 μ, were mounted in a dark room on " Kodak " lantern plates about twenty-four hours after the tissue had been removed from the patient. After the paraffin had been removed and the films developed, the tissue was stained *in situ* with hæmatoxylin and eosin.

At least five adjacent sections from each block of tissue were mounted on separate photographic plates and developed singly at intervals up to twenty days. The first radio-autograph from each block was developed after about twenty-four hours, and the times at which the other plates were subsequently developed were determined by the result of the initial radio-autograph. Most of the plates were developed at twenty-four hours, three days, seven days, fourteen days and nineteen days after the tissue was mounted.

INTERPRETATION OF RESULTS

The distribution of I^{131} could be correlated easily with, and appeared to be confined to, vesicles, however small. In view of the limitation of resolution inherent in the method, no attempt was made to define changes relative to individual cells (Forbes, 1951).

Used in the manner described, the method is roughly quantitative, in that the I^{131} content of nodular tissue can be compared with that of the unaffected adjacent tissue, and further, the duration of exposure required to produce a particular result gives a comparative estimate of the I^{131} content of the tissue. It may be

[1] Received on December 3, 1953.

[2] Now at Fairfield Hospital, Melbourne.

[3] This panel consists of a radiotherapist, a physician, a pathologist, a physicist and a biochemist. At the time when this material was collected, the constituent members were Dr. R. Kaye Scott or Dr. W. F. Holman, Dr. W. E. King, Dr. J. A. Forbes, Mr. K. H. Clarke and Mrs. D. Winikoff.

used to compare functional aspects of unaffected and diseased tissue of which a clinical assessment of the patient gives no indication.

The radio-autographs shown here demonstrate the capacity of the thyroid gland or carcinoma tissue to "take up" and retain iodine for periods of fifteen hours at least.

FINDINGS

In all the specimens examined, evidence of I^{131} uptake and storage was confined to vesicles; but even in normal thyroid not all vesicles showed equal evidence of iodine storage.

The various conditions found are described in two main groups, consisting of the various types of carcinomata on one hand and localized nodules on the other.

Carcinomata

Ten cases of carcinoma of the thyroid were examined, in all of which metastases were present variously in lymph nodes, lungs and bones, so that the diagnosis was not in doubt.

Three of the carcinomata were differentiated, in that the tissue contained numerous vesicles and resembled thyroid tissue; five were

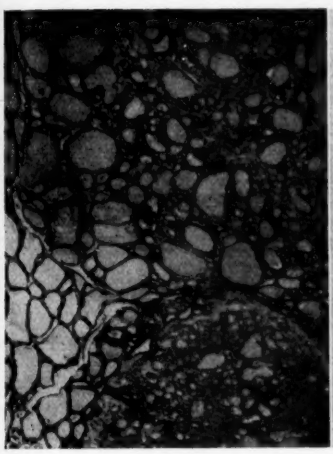

FIGURE I

An ordinary histological section showing the margin of a well-differentiated thyroid carcinoma. This area of tissue corresponds to that indicated by the arrow in Figure II and the radio-autograph in Figure III

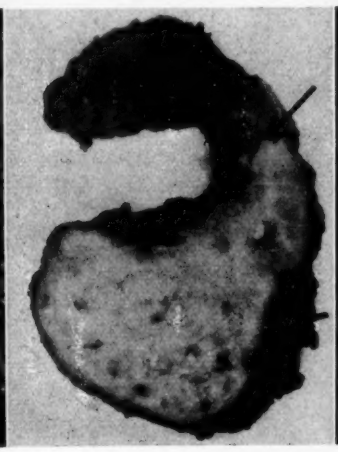

FIGURE II

Macroscopic appearance of the carcinoma shown microscopically in Figure I. The arrow indicates the site from which the microscopic section and radio-autograph in Figure III were taken

FIGURE III

Radio-autograph corresponding to the histological section in Figure I. Note the paucity of "uptake" of radio-active iodine by the tumour in contrast to the dense "uptake" by the uninvolved thyroid tissue. Tracer dose I^{131}: 300 μc. Exposure: seven days

There was a patchy variation in the concentration of I^{131} which generally appeared to be accentuated in pathological conditions except in involutionary tissue.

By contrast, tissue in which no vesicles were observed showed no evidence, under the conditions of this examination, of being able to store I^{131} in excess of non-thyroid tissue.

Well differentiated types of thyroid carcinomata, localized nodules consisting of involutionary tissue and nodules in which there was replacement of thyroid cells, as in Hashimoto's disease, contained vesicles in which the evidence of function was negligible when compared with that of normal thyroid tissue.

anaplastic uniformly cellular tumours consisting of either spindle-shaped or round cells, whilst the remaining two were intermediate in type, having a few vesicles scattered amongst papilliform or cellular tissue.

The I^{131} content of these tumours, whether differentiated or not, was greatly reduced below that of normal thyroid gland, and although the radio-autographs of the differentiated tumours did show some evidence of I^{131} storage, the amount present contrasted with that seen in the surrounding unaffected thyroid tissue (Figure III).

The radio-autographs of the anaplastic cellular tumours showed no evidence of I^{131} storage in excess of non-thyroid tissue (Figure IV), nor

did the intermediate type of tumours (Figure V). These intermediate or semi-differentiated forms were observed in patients in whom the thyroid gland was still present, and although the sparsely scattered vesicles showed no evidence of iodine storage, it has been reported that storage may be observed in this type of tumour after removal of the thyroid gland (Rawson *et alii*, 1948 ; Pochin *et alii*, 1952).

The orthodox histological criteria for diagnosis of malignant disease in other parts of the body were alone insufficient to distinguish the highly differentiated thyroid carcinomata from adenomata or papillomata. In two of the cases, by the use of ordinary microscopy, it was scarcely possible to discern the junction of tumour and thyroid tissue (Figure I). This junction, evident macroscopically (Figure II), was apparent in the radio-autograph, as the I^{131} content of the tissue was greatly reduced below that of the adjacent gland tissue (Figure III).

One of the intermediate forms, too, presented the orthodox histological appearances of a benign tumour ; but the presence of metastases left no doubt as to the diagnosis.

The anaplastic tumours presented the usual microscopic features of malignant change.

About 30 carcinoma transplants in rats,[1] of which half were differentiated forms of carcinoma, were examined. The same principles applied : namely, that I^{131} was stored only in vesicles, and even though the carcinoma tissue greatly resembled normal thyroid tissue in appearance, the concentration of I^{131} was much smaller than that seen under similar conditions in unaffected thyroid tissue (Figure VI).

Localized Nodules

Localized thyroid nodules were obtained at operation from 11 patients who had previously received a tracer dose of I^{131} ; in two of these patients carcinoma was coexistent. These nodules fell into five groups, which are distinguishable by histological and radio-autographic characteristics. The groups are referred to as (i) inactive involutionary nodules, (ii) active or hyperplastic nodules, (iii) degenerating active nodules, (iv) inactive cellular nodules, and (v) Hashimoto's disease, the term " active "

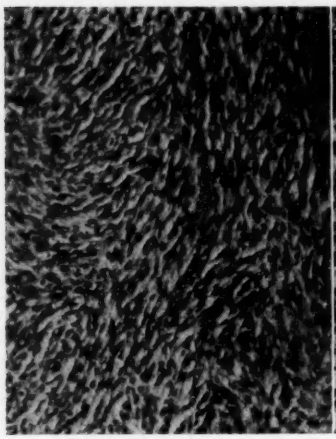

FIGURE IV

Undifferentiated spindle-cell carcinoma of thyroid gland. No I^{131} storage was observed in undifferentiated tumours of this type. Tracer dose I^{131} : 300 μc. Exposure: twenty-four days

FIGURE V

Radio-autograph of a papillary adenocarcinoma of the thyroid which has been classified as an intermediate form. Unlike that in Figure I, the vesicles are scanty and there is no evidence of " uptake " of radio-active iodine in the presence of the thyroid gland. Tracer dose I^{131} : 300 μc. Exposure: twenty-four days

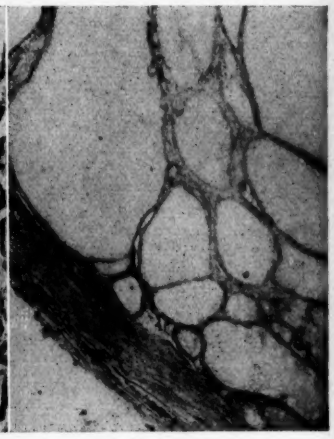

FIGURE VII

Radio-autograph of an involutionary nodule about five centimetres in diameter. Tracer dose I^{131} : 300 μc. Exposure : twenty-four days

[1] Work with the carcinoma transplants in rats was carried out in conjunction with Miss N. McQuillan and Mrs. P. Todd, of the Department of Biochemistry, University of Melbourne.

being used to describe tissue in which the "uptake" and storage of I^{131} are roughly equal to, or greater than, that seen in normal thyroid vesicles.

Inactive Involutionary Nodules. — The inactive involutionary nodule was obtained from five patients, and in one of these a carcinoma was coexistent. Macroscopically the nodules were circumscribed by condensed tissue. The cut surface was recognizable as thyroid, having the brownish translucent appearance produced by the presence of colloid.

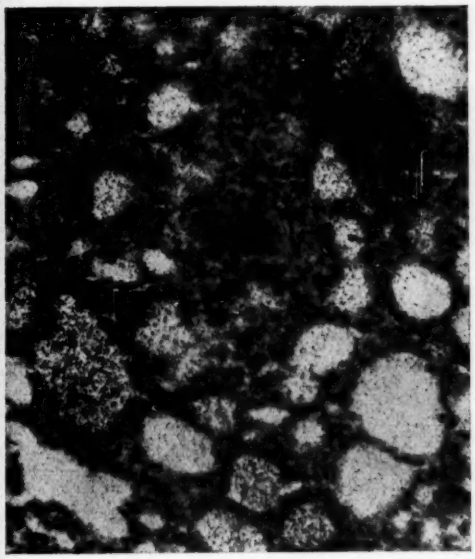

FIGURE VI

Radio-autograph of a well-differentiated carcinoma metastasis in a rat showing evidence of some I^{131} storage. Thyroidectomy had been performed, but the concentration of I^{131} in the tumour was much less than in normal rat thyroid gland

The nodules were multiple, often subdivided by bands of fibrous tissue, and occasionally contained areas of necrosis. Contact radio-autographs prepared from these demonstrated involutionary thyroid tissue consisting of large vesicles lined by flat epithelium surrounding pale colloid, in which there was very little evidence of I^{131} storage, even after exposure of the plates for twenty-four days (Figure VII). The unaffected thyroid tissue included in the sections showed evidence of uptake and storage after twenty-four hours' exposure. Coalescence of large vesicles had taken place in some of these and apparently represented one of the early stages of cyst formation.

Active or Hyperplastic Nodules.—Active or hyperplastic nodules were found in two patients, both of whom were suffering from intermittent thyrotoxic symptoms from recurrent goitre. Macroscopically, the cut surface had a heterogeneous appearance; in some areas it was fleshy, whilst in others colloid was apparent. Microscopically the radio-autographs showed evidence of hyperactivity, in that the amount of I^{131} taken up was in excess of normal in many areas (Figure VIII). In these areas the vesicles, which were lined by cubical epithelium, were generally smaller than normal and contained colloid which was vacuolated at the periphery. In places there were papillæ projecting into the lumina of the vesicles. This tissue was subdivided by bands of fibrous tissue (Figure VIII). No active nodules embedded in otherwise normal glands were found. These are uncommon (Means, 1951); but it seems feasible in a general classification to include this type of nodule here.

Degenerating Active Nodules. — Degenerating active nodules were found in three cases in which there was viable colloid-containing tissue usually at the periphery associated with tissue in various stages of necrosis. Macroscopically, central necrosis in the nodule was usual. Microscopically, the viable tissue present consisted of small, rather regular rounded vesicles separated by relatively large amounts of homogeneous acidophile material. At the periphery of these nodules the vesicles were more concentrated and showed radio-autographic evidence of relatively normal I^{131} storage. Towards the centre of the nodules the vesicles were sparser and showed diminishing evidence of function. The centres of these nodules consisted of tissue debris (Figure IX). Cysts which contained active thyroid tissue in their walls appear to be the end result of this necrotic process (King, 1952) (Figure X).

Inactive Cellular Nodules.—In two cases, nodules were found which differed in appearance and functional activity from the former groups. One of these nodules occurred in the same lobe as a well-differentiated carcinoma (Figure XI), the other in a recurrent goitre (Figure XII). In both these cases the nodules appeared to be localized. The diameter of the nodule associated with the carcinoma was two centimetres; that of the other, about one centimetre. Macroscopically they consisted of dense white tissue, which was not recognizable as thyroid tissue. Microscopically the nodules consisted

of densely packed cells, amongst which widely scattered irregular vesicles were present. The radio-autographs showed that the storage of I^{131} was slight and was confined in small amounts to occasional vesicles (Figures XI and XII). The only tissue showing analogous histological and functional characteristics to this was neoplastic thyroid tissue. One of these nodules being situated in the same lobe as a carcinoma was probably receiving neoplastic stimulation.

Hashimoto's Disease. — Hashimoto's disease was represented by one case. The pathological changes were confined mainly to one lobe, and clinically the condition was difficult to distinguish from other types of nodular goitre, particularly carcinoma. Macroscopically, the affected lobe, which was about eight by five by three centimetres in size, consisted of numerous rounded, pale, fleshy nodules separated by fibrous bands. Microscopically, evidence of I^{131} uptake and storage was confined to the few vesicles which remained in the mass of round cells characteristic of the disease (Figure XIII).

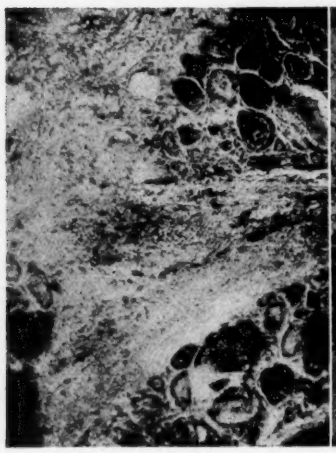

FIGURE VIII

Radio-autograph of a hyperplastic recurrent thyroid nodule in a woman with thyrotoxicosis. Tracer dose I^{131}: 100 μc. Exposure: twenty-four hours

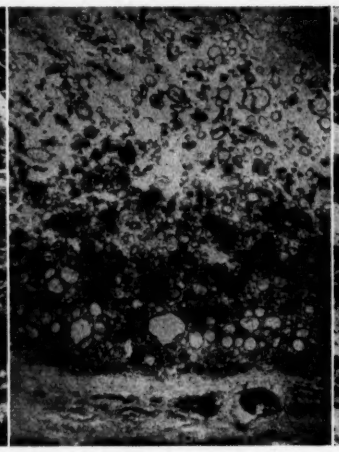

FIGURE IX

Radio-autograph of an "active" nodule in which there is central necrosis. Tracer dose I^{131}: 100 μc. Exposure: three days

FIGURE X

Wall of a solitary cyst about five centimetres in diameter containing "active" thyroid tissue. Tracer dose I^{131}: 100 μc. Exposure: six days

DISCUSSION

Functional Activity in Thyroid Carcinomata

Statistical studies on nodular goitre in which thyroid function is related to the diagnosis of carcinoma provide a background for the radio-autographic observations of thyroid tumours.

These observations, which are concerned with the function of the gland as a whole, indicate that the association of toxicity with thyroid carcinoma is unusual, and that the production of toxic symptoms is probably not a function of thyroid carcinomata (Cole *et alii*, 1945; Beahrs *et alii*, 1951).

Means (1948, 1951) has observed that the function of malignant tumours of the thyroid gland gauged by their capacity to collect I^{131} and determined by Geiger counting, is much below that of normal uninvolved thyroid tissue. The clinical corollary of this has been that inactive nodules which do not metabolize I^{131} or metabolize it in negligible amounts are suspect as carcinomata, whereas the chances of "active" nodules being carcinomata are negligible.

The precise relationship of the amount and type of tissue with its capacity to metabolize iodine is difficult to discern by Geiger counting methods, but when these are considered in conjunction with the radio-autographic appearances more precise conclusions are possible.

Evidence of I^{131} uptake and storage is found only in tumours in which vesicles are present (Figures III and VI), and in these the I^{131} is confined to the vesicles.

The highly differentiated tumours in which the tissue present closely resembles normal thyroid tissue may show evidence of I^{131} uptake and storage, even though the thyroid gland is still present (Figure III), but they do not metabolize I^{131} in amounts comparable with normal thyroid tissue even after the thyroid gland has been removed, and the concentration of I^{131} in these tumours is many times less than in normal thyroid tissue (Figure III). This confirms the opinion of Cole, Slaughter and Rosseter (1945) that in those cases in which carcinoma of the thyroid and thyrotoxicosis coexist, the overactivity occurs in the gland apart from the neoplasm.

fraction of that of normal tissue, as indicated by the time of exposure required to produce the radio-autographs (Figure III). This conforms with the large series referred to above, and appears to indicate that although a great volume of metastatic carcinoma tissue producing small quantities of thyroxin per unit mass may be capable of preventing myxœdema, its functional capacity per unit mass will almost inevitably be negligible in comparison with normal thyroid.

This discrepancy between the histological appearance of highly differentiated carcinoma tissue which closely resembles normal thyroid tissue and its inability to metabolize iodine

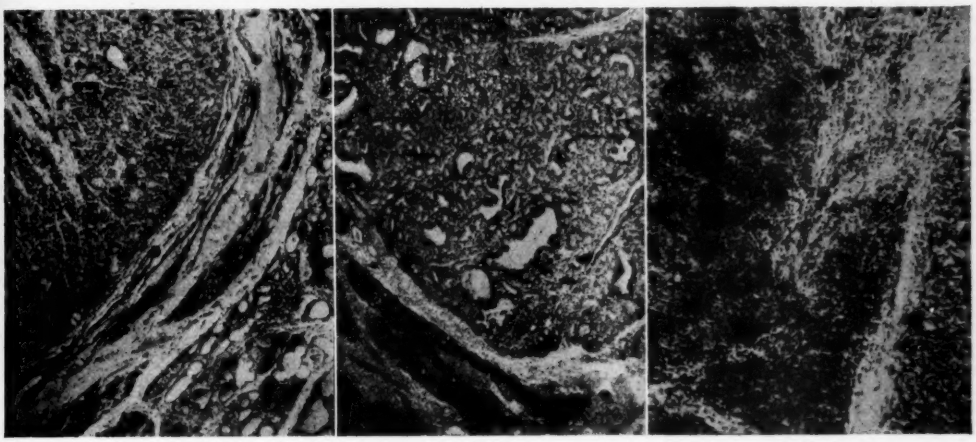

FIGURE XI

Radio-autograph showing the margin of a cellular nodule in which the "uptake" of I^{131} has been negligible by comparison with the neighbouring uninvolved thyroid tissue. This nodule was associated with a well-differentiated carcinoma. Tracer dose I^{131}: 300 μc. Exposure: seven days

FIGURE XII

Radio-autograph of a relatively cellular nodule containing irregular vesicles which show no evidence of I^{131} storage in contrast to the adjacent thyroid tissue. This nodule, which occurred in a recurrent goitre, was about one centimetre in diameter. Tracer dose I^{131}: 100 μc. Exposure: ten days

FIGURE XIII

Radio-autograph of thyroid gland affected with Hashimoto's disease. Tracer dose I^{131}: 100 μc. Exposure: three days

Some authors appear to retain the mental reservation that the highly differentiated forms of carcinoma may function as well as normal thyroid tissue. This is based upon the observations that patients with well-differentiated carcinomata do not become myxœdematous after the thyroid gland is removed. Although the highly differentiated carcinoma tissue examined here from the three human subjects and from 15 carcinoma transplants in rats, was in places almost indistinguishable histologically from normal thyroid tissue, the ability of the tissue to concentrate I^{131} was only a very small

when compared to normal thyroid tissue, may possibly be explained by two hypotheses: first, that the individual carcinoma cells are incapable of metabolizing iodine as efficiently as the unaffected cells; secondly, that unlike the normal gland, in which the circulation is ideally arranged for the clearance of iodine from the blood, the circulation of the tumours is inadequate in this respect.

The semi-differentiated tumours examined here, which consist of tissue containing sparsely scattered, ill-formed vesicles, show no evidence of being able to take up and store I^{131} under

the conditions prevailing (Figure V), although it has been reported that this type of tumour may show evidence of I^{131} storage after removal of the thyroid gland.

Uniformly cellular anaplastic tumours show no evidence that they are able to metabolize I^{131} in excess of non-thyroid tissue (Figure IV).

Recognition of Thyroid Neoplasms

The majority of thyroid neoplasms consist of relatively undifferentiated cells arranged in a disorderly fashion, and may be recognized by their histological appearances; but the early recognition of carcinomata which consist of thyroid-like tissue may be impossible on histological grounds alone.

The patients with differentiated tumours here were all in an advanced stage, and the fact that ordinary microscopic delineation was scarcely possible in these demonstrates the improbability of making the diagnosis in an early stage.

The relative deficiency in function observed by radio-autography appears to be so constant that it may be used as a criterion for the diagnosis and delineation of early thyroid-like neoplasms which might otherwise be diagnosed as hyperplasia. Conversely, the diagnosis of hyperplasia requires the demonstration of the capacity of the tissue of a nodule to metabolize I^{131} in amounts roughly equal to, or greater than, that of normal tissue.

Differentiation of Benign and Malignant Neoplasms.—It is well known that the criteria for the diagnosis of benign neoplasms of the thyroid gland are uncertain (Willis, 1948).

The relatively frequent occurrence of primary thyroid carcinomata which are so highly differentiated that the histological appearance is "benign", and which appear to be well localized even though metastases have already occurred, indicates that the histological segregation of benign from early malignant tumours is scarcely feasible.

Consequently, in the absence of criteria for the recognition of benign neoplasms, and in view of the variation in the life history of the carcinomata, particularly with regard to the time at which metastases become apparent, the separation of benign and malignant categories of thyroid neoplasms does not appear to be justified.

The Diagnosis of Nodular Goitre

The various types of nodules make up two broad functional groups: one is comprised of nodules in which concentration of I^{131} is roughly equal to, or greater than, that of normal thyroid tissue, or active nodules; the other consists of nodules in which the concentration of I^{131} is negligible in comparison with normal thyroid tissue, or inactive nodules. Although these groups can be segregated at times by Geiger counting methods, the ultimate diagnosis rests upon the histological appearance of the tissue in conjunction with evidence of its capacity to metabolize iodine. The technique of radio-autography as described here is sufficiently simple to warrant routine use in the investigation of nodular goitre in institutions where the facilities are available, and appears to provide a more certain method of determining the nature of the nodule.

Active Nodules.—The "active nodule" group consists of nodules in which the viable tissue present is capable of metabolozing iodine in amounts comparable to, or greater than, normal tissue. It includes hyperactive nodules, active nodules in which degeneration or necrosis is occurring and nodules which have become cysts with active tissue in their walls. All these are considered broadly as dyscrasias.

Inactive Nodules.—The viable tissue of nodules in the "inactive nodule" group demonstrates the capacity to store I^{131} in only negligible amounts. It includes carcinomata, involutionary nodules, Hashimoto's disease and a small group of relatively cellular, apparently localized, nodules which on the criteria appearing here are also carcinomata. These inactive cellular nodules, which may be inconspicuous, could well be diagnosed as hyperplasias in the absence of radio-autographic evidence. This group consists of both carcinomata and dyscrasias.

Comment.—It can be seen that the groups differentiated by function for diagnostic purposes do not coincide with the ætiological types, and that ætiologically the various nodules appear to fall into only two main groups, malignant neoplasms on one hand and dyscrasias on the other.

SUMMARY

Nineteen cases of nodular goitre, including 11 cases of carcinoma of the thyroid, have been studied by combined histological and radio-autograph techniques. Studies were also made in carcinoma transplants in rats.

Radio-autographs were prepared from specimens taken at operation fifteen hours after a dose of I^{131}.

I^{131} was retained only in tissue in which vesicles were present, and was confined to the vesicles themselves.

The functional activity of thyroid carcinomata is discussed.

Evidence is advanced for the ætiological classification of thyroid nodules in two main groups only, carcinomata and dyscrasias.

The nodules fall into two main functional groups. The first, showing evidence of normal or excessive iodine uptake, comprised hyperplastic nodules and degenerating and cystic hyperplastic nodules. Evidence is shown that this group is unlikely to be neoplastic. The second group, showing little or no evidence of iodine uptake, comprised various types of carcinoma, inactive involutionary nodules, a case of Hashimoto's disease and inactive cellular nodules which on the evidence presented are also carcinomata.

ACKNOWLEDGEMENTS

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"SECONAL" SEDATION TEST IN ARTERIAL HYPERTENSION: LIMITATIONS OF ITS VALUE[1]

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Cady, Horton and Adson (1936) used "Sodium Amytal", "Pentothal Sodium" and sodium and amyl nitrite in the investigation of hypertensive patients to estimate the fall in blood pressure to be expected from extensive sympathectomy. Allen and Adson (1938), in assessing hypertensive patients prior to sympathectomy, noted the change in blood pressure following the oral administration of 0·2 gramme (three grains) of "Sodium Amytal" for three successive hourly periods, and also to the slow intravenous injection of a 5% solution of "Pentothal Sodium". They stated that "when the diastolic pressure decreased to less than 110 (mm. Hg.) as a result of the oral administration of 9 grains (0·6 gm.) of Sodium Amytal divided in three hourly doses, good or fair results occurred almost four times as frequently as when the diastolic blood pressure decreased to a figure greater than 120". They also stated that "unfortunately for accuracy of preoperative prediction, there were many instances of eventual poor effects of operation on blood pressure in which the response of the diastolic blood pressure to the measures mentioned was entirely adequate". The "Sodium Amytal" test is inconvenient in practice. Sleep may not be produced in a reasonable time, and the patient may be unduly drowsy on the following day.

Frew and Rosenheim (1949) modified the test by substituting the rapidly acting barbiturate "Seconal". Their method was as follows. With the patient lying in a quiet room the initial blood pressure was recorded; "Seconal" was then given in a draught of water, and the blood pressure was determined at five-minute intervals until a "base-line" was obtained or the patient awakened. A dose of 4·5 grains usually produced sleep, but occasionally this was inadequate and 6·0 grains had to be given on another occasion. These workers compared the effect of the intravenous injection of tetra-ethyl ammonium bromide (TEAB)—usual dose 500 milligrammes—and a "Seconal" sedation test in the investigation of patients with arterial hypertension. They found that the minimum diastolic blood pressure resulting from the intravenous injection of TEAB was usually similar to that resulting from a "Seconal" sedation test. Thus 29 of 38 patients tested with the two drugs showed a difference of 10 millimetres of mercury or less between the two minimum diastolic blood pressures. Also, in seven cases, TEAB was injected during "Seconal" hypnosis. In only one subject (with malignant hypertension) was a minimum diastolic pressure recorded "considerably lower" than that obtained with either drug alone. Frew and Rosenheim suggested that both drugs acted upon the "neurogenic element" of hypertension, though at different levels of the reflex arc.

We found the "Seconal" test easier to perform than the older "Sodium Amytal" test. With the introduction of the methonium compounds as ganglionic blocking agents, we considered that it would be interesting to compare the effect of the injection of these compounds and of the "Seconal" sedation test on blood pressure in hypertensive subjects in a fashion similar to that already done by Frew and Rosenheim with TEAB and "Seconal". We thought that confirmatory evidence might thereby be obtained concerning the presence of a "neurogenic element" in hypertension. We were disappointed in this hope, but consider that our experiences with the "Seconal" test are worth reporting in case this or another barbiturate sedation test is being used elsewhere.

Materials and Methods

Material

The observations were made on 19 patients with severe arterial hypertension, 10 with and nine without papillœdema. Hexamethonium bromide, two milligrammes per kilogram of body weight, was given by deep intramuscular injection. The "Seconal" test was performed

[1] Received on December 2, 1953.

in the manner described by Frew and Rosenheim; 17 patients received 4·5 grains, the other two patients 6·0 grains.

The " Seconal " and hexamethonium tests were carried out on different days.

Recording

Blood pressure and pulse rate were recorded at five-minute intervals for thirty minutes before the methonium injection or " Seconal " administration and then until a " floor " had been established, or (in the case of the " Seconal " test) until the patient awakened. A mercury column type of sphygmomanometer was used. The diastolic blood pressure was recorded at the disappearance of sound.

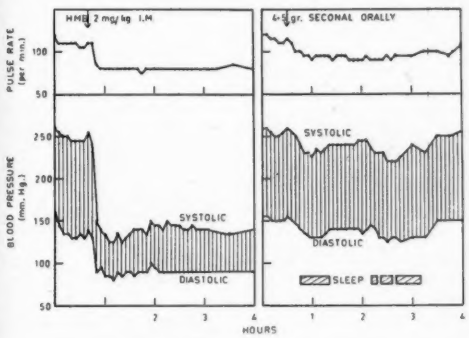

FIGURE I

Results from intramuscular injection of hexamethonium bromide and a " Seconal " sedation test in the same patient

As we did not wish to place too much reliance on a single blood pressure reading, the initial base-line blood pressure was taken as the average of five readings before the administration of the methonium or " Seconal ", and the " floor " as the average of five readings when the blood pressure was at its lowest. In some of the " Seconal " tests no satisfactory " floor " was established, as immediately after reaching its lowest point the blood pressure began to rise. In these instances the " floor " was taken as the average of the lowest reading and those immediately before and after it.

RESULTS

Although the fall in systolic and diastolic blood pressure from the intramuscular injection of hexamethonium bromide varied from patient to patient, the type of response was fairly uniform. The blood pressures fell to their lowest level within fifteen minutes; there was a fairly steady level for a period of an hour or more, and then a gradual rise to the pre-injection level (Figure I).

In the " Seconal " sedation tests the responses were varied. In only a few instances was there a " floor " in the sense that it occurred with methonium (Figure IIA). In other instances there was a sharp fall and then sharp rise

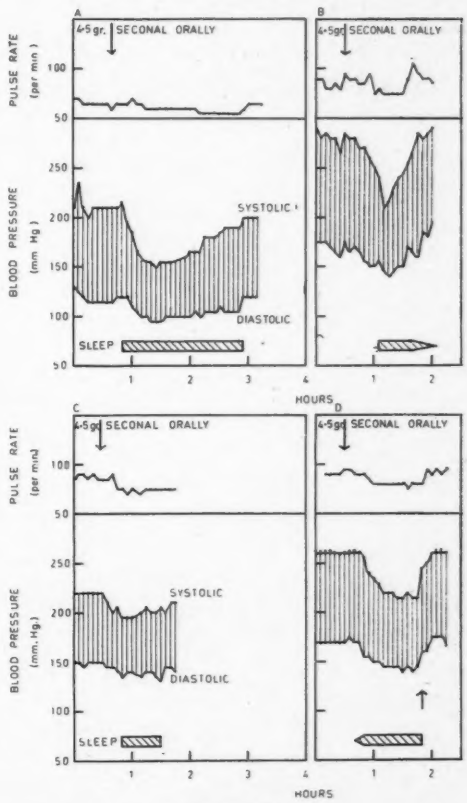

FIGURE II

Results of " Seconal " sedation tests in four different patients, showing some of the types of response. At the time indicated by the lower arrow in D, the patient awoke suddenly

(Figure IIB), a sharp fall and gradual recovery, or a " slump " (Figure IIC). The fall in blood pressure was apparently related to the sleep induced, as indicated by the sharp rise in blood pressure in some subjects on awakening (Figure IID).

The experimental observations are recorded in Table I. The marked differences both in the extent of the blood pressure fall and in the " floor " levels from the two tests are apparent.

These are shown for one patient in Figure I. The percentage blood pressure falls from the two tests in all the patients are compared in Figure III and the blood pressure "floors" in Figure IV.

DISCUSSION

Our findings with "Seconal" and hexamethonium bromide are in such marked contrast to those with "Seconal" and TEAB (Frew and Rosenheim, 1949) that we think there must be some simple explanation.

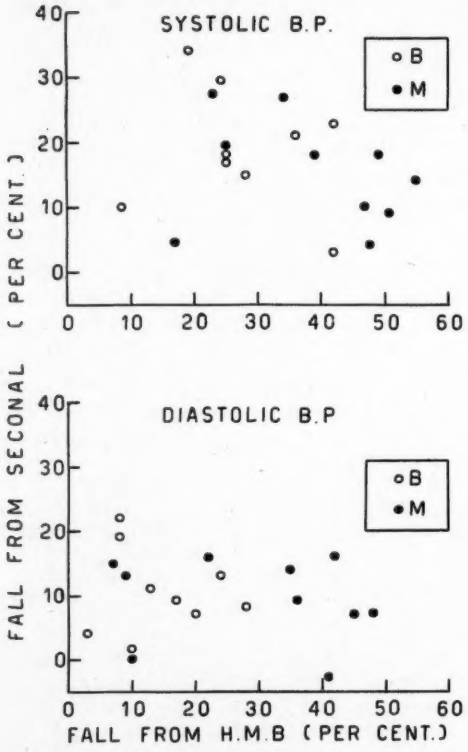

FIGURE III

Comparison of percentage falls in systolic and diastolic blood pressure from "Seconal" and hexamethonium bromide tests on 19 patients; B, benign; M, malignant

The first possibility is that the differences are due to the subjects studied. In the study quoted above, five of the 38 patients were in the malignant phase; in our series 10 of the 19 patients were in this phase. Approximately a third of their patients, but none of our patients, had an initial diastolic blood pressure below 109 millimetres of mercury. It is therefore apparent that, considered as a whole, our patients had more severe hypertension.

The second possibility was that the tests were not comparable. The "Seconal" sedation test was carried out in the same manner in the two investigations. However, the effect of ganglionic blockade with hexamethonium bromide may not be comparable with that of TEAB.

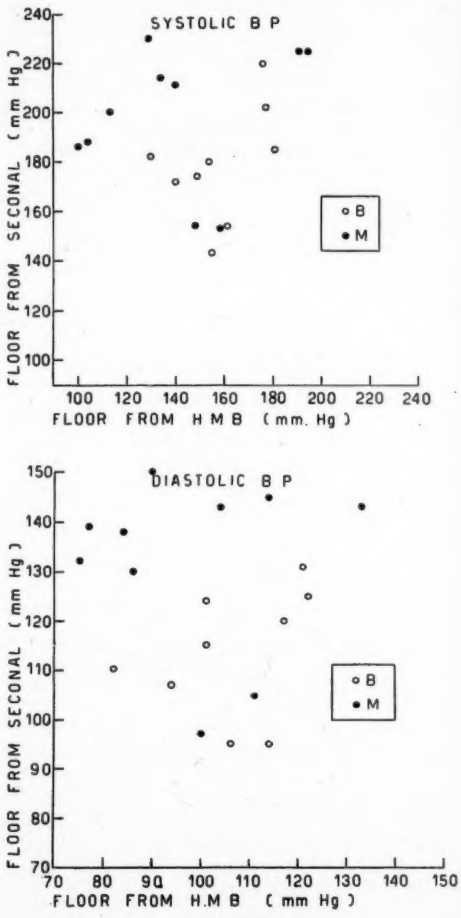

FIGURE IV

Comparison of systolic and diastolic blood pressure "floors" from "Seconal" and hexamethonium bromide tests; B, benign; M, malignant

In Table II we have compared the blood pressure changes in our series with those found by the previous workers in patients with roughly comparable degrees of hypertension. The range of falls in both series is wide. The average blood pressure fall observed by us in the "Seconal" test was about a half that observed by Frew and Rosenheim. The average fall

TABLE I

Comparison of Changes in Blood Pressure and Pulse Rate After the "Seconal" Sedation Test and the Intramuscular Injection of Hexamethonium Bromide (Two

TABLE I

Comparison of Changes in Blood Pressure and Pulse Rate After the "Seconal" Sedation Test and the Intramuscular Injection of Hexamethonium Bromide (Two Milligrammes per Kilogram)

Case No.	Sex	Age (Yrs.)	Diagnosis[1]	Methonium Test									"Seconal" Sedation Test								
				Initial Base-line Values			"Floor" Values			Percentage of Change in Initial Base-line Values			Base-line Values			"Floor" Values			Percentage of Change in Base-line Values		
				Blood Pressure (Millimetres of Mercury)		Pulse Rate (per Minute)	Blood Pressure (Millimetres of Mercury)		Pulse Rate (per Minute)	Blood Pressure (Millimetres of Mercury)		Pulse Rate (per Minute)	Blood Pressure (Millimetres of Mercury)		Pulse Rate (per Minute)	Blood Pressure (Millimetres of Mercury)		Pulse Rate (per Minute)	Blood Pressure (Millimetres of Mercury)		Pulse Rate (per) Minute)
				S.[2]	D.[2]		S.	D.		S.	D.		S.	D.		S.	D.		S.	D.	
1	F.	52	E (M)	231	126	87	191	114	78	−17	−10	−10	235	145	90	225	145	85	−4	0	−6
2	M.	39	N (M)	205	120	77	159	111	67	−23	−7	−13	211	124	75	155	105	60	−26	−15	−20
3	F.	46	E (M)	246	132	107	129	86	80	−47	−35	−25	254	151	113	230	130	94	−9	−14	−17
4	F.	51	E (B)	219	118	81	140	94	80	−36	−20	−1	218	115	70	172	107	74	−21	−7	+6
5	F.	59	E (B)	246	133	72	176	101	57	−28	−24	−21	260	142	77	220	124	60	−15	−13	−22
6	M.	53	E (M)	225	129	72	148	100	75	−34	−22	+4	210	115	65	154	97	60	−27	−16	−8
7	F.	39	E (B)	191	115	85	155	106	94	−19	−8	+11	201	117	74	143	95	70	−34	−19	−5
8	F.	38	E (B)	236	140	78	177	122	69	−25	−13	−12	247	140	65	202	125	65	−18	−11	0
9	F.	43	E (M)	257	146	83	194	133	84	−25	−9	+1	277	164	84	225	143	70	−19	−13	−17
10	M.	37	E (M)	231	145	92	104	77	79	−55	−48	−14	220	150	95	189	139	93	−14	−7	−2
11	F.	52	E (B)	224	114	92	130	82	86	−42	−28	−7	237	119	89	182	110	73	−23	−8	−18
12	F.	50	E (M)	262	180	92	134	104	77	−49	−42	−16	262	170	91	216	143	78	−18	−16	−14
13	M.	46	E (M)	189	127	100	98	75	78	−48	−41	−22	194	130	100	186	132	94	−4	+2	−6
14	F.	40	E (B)	213	124	93	161	114	111	−24	−8	+19	220	122	121	156	95	91	−29	−22	−25
15	M.	49	E (B)	200	121	80	149	101	78	−25	−17	−2	212	127	70	176	115	76	−17	−9	+9
16	M.	63	E (M)	233	154	85	113	84	67	−51	−45	−21	220	148	86	200	138	75	−9	−7	−14
17	M.	42	E (B)	200	125	70	181	121	70	−9	−3	0	206	137	69	185	131	66	−10	−4	−4
18	M.	37	E (M)	230	140	86	140	90	90	−39	−36	+5	257	165	81	212	150	65	−18	−9	−20
19	M.	35	E (B)	205	130	85	154	117	85	−42	−10	0	185	120	80	180	120	80	−3	0	0

[1] E, essential; N, nephritic; B, benign; M, malignant.
[2] S, systolic; D, diastolic.

TABLE II

Comparison of Blood Pressure Changes from Hexamethonium Bromide and "Seconal" (Present Series) with those from TEAB and "Seconal" (Frew and Rosenheim)

Hexamethonium Bromide and "Seconal"							TEAB and "Seconal"						
Initial Diastolic Blood Pressure (Millimetres of Mercury)	Drug	Number of Patients	Average Fall in Diastolic Blood Pressure (Millimetres of Mercury)	Range of Fall	Average Percentage Fall in Diastolic Blood Pressure	Range of Percentage Fall.	Initial Diastolic Blood Pressure (Millimetres of Mercury)	Drug	Number of Patients	Average Fall in Diastolic Blood Pressure (Millimetres of Mercury)	Range of Fall	Average Percentage Fall in Diastolic Blood Pressure	Range of Percentage Fall
110–129	Hexamethonium bromide	10	20	4 to 52	16	3 to 41	110–129	TEAB	13	31·6	13·6 to 65	26·6	11 to 57
130 and over		9	43	13 to 76	29	9 to 48	130 and over		21	30·3	6 to 48	21·0	5 to 36
110–129	"Seconal"	8	14	0 to 27	12	0 to 22	110–129	"Seconal"	15	25·6	10 to 42	20·1	8 to 36
130 and over		11	13	−2[1] to 27	8	−2[1] to 16	130 and over		19	30·3	10 to 48	19·6	7 to 37

[1] A rise in blood pressure is shown as a negative fall.

produced by hexamethonium bromide was less than that produced by TEAB in the patients with an initial diastolic blood pressure of 110 to 129 millimetres of mercury, and greater than that produced by TEAB in those with an initial diastolic blood pressure of 130 millimetres of mercury and over.

As our results differ even with the same test ("Seconal"), we consider that the main reason for the discrepancy is in the difference in the type of subject.

Because of our experiences with "Seconal" and hexamethonium bromide, we have looked critically at the results of Frew and Rosenheim with "Seconal" and TEAB. Although in many instances the "floor" produced by the two drugs was approximately the same, in nine out of the 38 cases (that is approximately one-quarter) there was a difference of more than 10 millimetres of mercury between the minimum diastolic blood pressures. The importance of a correlation between minimum diastolic blood pressures obtained from "Seconal" and from TEAB may be over-estimated. Such a correlation would be expected if the initial diastolic blood pressures showed a wide range. The best correlation would occur if there were no change from the administration of the drugs.

The difference in "floors" obtained in our results suggests that either (*a*) the two drugs reduce blood pressure by different mechanisms, or (*b*) that while they act by the same mechanism the quantitative effects are different. Paton and Zaimis (1948, 1949) found that hexamethonium bromide acted by ganglionic blockade, and although much work has since been done with these drugs, no other action has been demonstrated.

Views of pharmacologists on the importance and mechanism of the vascular effect of the barbiturate drugs differ. Sollman (1948) states:

> Anæsthetic doses cause no significant change in the circulation, clinically or in animals, with oral, hypodermic or slow intravenous administration. The blood pressure remains normal . . .

Krantz and Carr (1949) state:

> In oral therapeutic doses sufficient to produce sedation or sleep the barbiturates *do not significantly affect the circulation*. The blood pressure remains normal . . . Large doses of barbiturates produce peripheral vasodilatation and a fall in blood pressure.

The blood pressure lowering and peripheral vasodilator actions of the barbiturate drugs have been established experimentally (Gruber and Baskett, 1925; Gruber, 1941; Richter and Oughterson, 1932). The mechanism of these effects is apparently complex, but they are commonly believed to act at a brain stem level. Thus Gaddum (1948) states:

> Barbiturates are particularly liable to affect the autonomic system. They have a relatively powerful depressant action on the hypothalamic centres which control autonomic activities, and various peripheral effects which have not been systematically studied.

Exley (1952) described a depressant action of the barbiturates on autonomic ganglia. Other workers (Gruber and Roberts, 1926) have described a vasodilator effect on perfused vessels. There is experimental evidence that the barbiturates may have a cardiac action, either by a toxic effect on the cardiac vagus nerve (Gruber and Keyser, 1946) or directly on the heart muscle (Roth, 1935; Gruber and Keyser, 1946).

TABLE III

Response to Treatment Compared with the Results of "Seconal" and Hexamethonium Bromide Tests

Test and Response	Response to Methonium Treatment			
	Good	Fair	Poor	Total
"Seconal" test:				
Good	0	0	1	1
Fair ..	1	0	2	3
Poor	7	1	3	11
Total ..	8	1	6	15
Hexamethonium bromide test:				
Good	5	1	2	8
Fair ..	1	0	0	1
Poor	2	0	4	6
Total ..	8	1	6	15

With lack of precise knowledge of the vascular effects of the barbiturates, it would seem unprofitable to discuss the possibility of different quantitative autonomic blockade from methonium compounds and from barbiturates. It would also seem unwise to draw firm conclusions on the mechanism of hypertension from the action of the latter drugs.

We have not found the "Seconal" sedation test useful in predicting the response to treatment by sympathetic blockage. Fifteen of the subjects who were given "Seconal" and hexamethonium bromide tests later received continuous treatment with the methonium compounds. For purpose of brief description the responses to the tests and to treatment have been arbitrarily classified as "good", "fair" and "poor". (In a "good" response to a test, the diastolic blood pressure fell to 110 millimetres of mercury or below and by 20% or more; in a "poor" response, neither

of these criteria was fulfilled; and in a " fair " response one only was fulfilled.)

The response to the tests and to treatment is compared in Table III. Seven patients who gave a poor response to the " Seconal " test gave a good response to treatment, and one patient who gave a good response to this test gave poor response to treatment. (The correlation between the result of the hexamethonium test and the response to treatment, although somewhat better, was still not very good.)

SUMMARY AND CONCLUSIONS

The hypotensive effect of a " Seconal " sedation test and of the intramuscular injection of hexamethonium bromide (two milligrammes per kilogram) was compared in 19 patients with severe hypertension (11 in the " malignant " and nine in the " benign " phase).

There was no appreciable correlation between the percentage blood pressure falls or the blood pressure " floors " from the two procedures.

These findings are discussed. It is concluded that the discrepancy between our findings and those of other workers who used a " Seconal " test and then injection of TEAB may be most likely explained by the difference in type of patient.

However, because of uncertainty concerning the mechanism of production of vascular effects by the barbiturate drugs, we believe that conclusions on the mechanism of hypertension based on the findings of the " Seconal " test should be regarded with suspicion.

The " Seconal " sedation test is probably valueless in predicting the response to treatment by sympathetic blockade.

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THE MECHANISM OF ARTERIAL HYPERTENSION : A COMPARISON OF THE EFFECTS OF HEXAMETHONIUM BROMIDE IN HYPERTENSIVE AND NORMOTENSIVE PERSONS[1]

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The mechanism of human arterial hypertension is still not fully elucidated, although it is commonly believed to involve an increased peripheral resistance arising from a functional narrowing of the systemic arterioles. This view is based on the evidence of many observers which may be summarized as follows. An increased blood pressure could occur from either an increased volumetric cardiac output, or an increased resistance ; but as the cardiac output is normal (Goldring and Chasis, 1947) the peripheral resistance must be increased. This may be due to an increased viscosity of the blood or to an altered configuration of the vessels (Poiseuille's law, 1843). As, however, the viscosity of the blood in hypertensive persons is normal (Pickering, 1936), the increased resistance must be due to changes in the calibre of the systemic arterioles, since these vessels contribute most to the peripheral resistance. This vascular narrowing is probably not structural—at least in the early stages—for fever can reduce the peripheral resistance to normal (Bradley *et alii*, 1945). No pathological changes have been demonstrated in the renal vessels in early cases of hypertension (Castleman and Smithwick, 1943), but this is not surprising, as the narrowing would be very small and very careful observation would be necessary to detect it.

The possible causative agents of the postulated functional narrowing of the blood vessels in arterial hypertension may be grouped as follows : humoral, nervous and intrinsic. Early work was held to exclude the sympathetic nervous system in the production of arterial hypertension. Despite great effort to discover a humoral agent, none has been firmly established. The question of a possible " intrinsic " abnormality of the vessels leading to an increased responsiveness to normal stimuli will be discussed later.

If the increased vascular resistance in essential hypertension is due to sympathetic nervous over-activity, one would expect in subjects with this condition, (*a*) that the blood flow would be less than that of normotensive persons in a part where the vessels are normally under strong sympathetic vasoconstrictor influence and greater in a part in which this influence is minimal or absent, and (*b*) that on the removal of local sympathetic tone there would be an increase in blood flow greater than that occurring in persons with normal pressures. Early investigators (Prinzmetal and Wilson, 1936 ; Pickering, 1936 ; Stead and Kunkel, 1940) found that in the hand, foot and forearm the resting blood flow was the same in hypertensive as in normotensive persons. Also, after the vasomotor nerves had been blocked with an anæsthetic agent, or another part of the body had been heated (a procedure believed to release sympathetic tone), the blood flow in the regions mentioned increased by the same amount in the two groups. They believed that this evidence was against the participation of the sympathetic nervous system in essential hypertension.

However, subsequent work has led to a questioning of this conclusion on two grounds : (*a*) other investigators have made observations conflicting with those of earlier workers ; (*b*) the assumption that the vessels of muscle have very little sympathetic control is probably not true.

Abramson and Fierst (1942) measured resting blood flows in 70 hypertensive and 90 normotensive subjects. They found that in the forearm and muscular segment of the leg the blood flow was greater in hypertensive subjects, but in the hand the average blood flow was greater in normotensive subjects. Wilkins and Eichna (1941) found that following vasodilatation (by reactive hyperæmia) the muscle blood flow was higher in hypertensive than in normotensive persons.

Recent work (Barcroft and Swan, 1953) has indicated that there is marked sympathetic tone in muscle vessels, shown by the increase in blood flow following sympathetic interruption.

[1] Received on January 7, 1954.

There is much evidence that the sympathetic nervous system is concerned at least partially with the increased blood pressure in essential hypertension. Thus lumbodorsal sympathectomy causes a marked to moderate lowering of blood pressure in many hypertensive persons (Smithwick, 1948). The blood pressure may also be lowered by the administration of ganglionic blocking agents, such as the methonium compounds (Smirk and Alstad, 1951). The peripheral sympathetic blocking agent " Dibenamine ", while having little effect on the blood pressure in normotensive persons, produces a definite fall in the pressure in hypertensive persons (Haimovici and Medinets, 1948).

It may be argued that if any fall in blood pressure occurs from the administration of a " hypotensive " drug, this fall may be expected to be higher in hypertensive than in normotensive subjects, because of the higher base-line value in the former. However, in the observations of Haimovici and Medinets, the decrease in systolic and diastolic blood pressure from the administration of the sympatholytic drug was considerably greater in the hypertensive than in the normotensive group, even when expressed as a percentage of original level (our calculations).

It seems therefore that human arterial hypertension is most probably due to an increased peripheral resistance, and that there is some evidence for the participation of the sympathetic nervous system in producing this increased resistance. The studies already reported on the effect of sympatholytic agents in hypertensive persons have had certain shortcomings: the effect on the average blood pressure or the diastolic blood pressure was considered, with neglect of the effect on the systolic blood pressure; the changes were expressed in millimetres of mercury instead of as a percentage of the base-line value (the latter method is preferable, as the same absolute change in blood pressure may represent widely differing proportional changes owing to difference in base-line values); the dose of the sympatholytic drug was usually fixed, with no allowance for body weight, and no account was taken of changes in pulse rate coincident with changes in blood pressure.

PRESENT INVESTIGATION

As the introduction of new ganglionic bocking agents—the methonium compounds—has made it possible to ablate sympathetic nervous activity with reasonable safety, we decided to study the effect of sympathetic nervous blockade in hypertensive and normotensive persons, avoiding the defects in previous investigations. The pulse rate was recorded at the same time as the blood pressures. The methonium compounds block the transmission not only of sympathetic but also of parasympathetic impulses, and this action has to be considered in interpreting the observations.

The effect of intramuscular injection of hexamethonium bromide on the blood pressures and pulse rate in the hypertensive subjects is shown in Table I and in the normotensive subjects in Table II; a statistical analysis of the results is given in Table III. These show the following features.

Blood Pressures

There was a greater average fall in both the systolic and diastolic blood pressures in the hypertensive than in the normotensive subjects, both when the falls were expressed as an absolute value and when they were expressed as a percentage of the base-line (Figure I). The differences between the mean percentage falls of both the systolic and the diastolic pressures are statistically significant at the 1% level.

Considered as a whole, the hypertensive and normotensive groups were not strictly comparable with respect to age. However, if we consider only those subjects aged thirty to forty-nine years, the difference in response previously noted is still apparent (Figure I, shaded sections). If a correction is applied for age (see later), the differences in the response of the two original series are still statistically significant at the 1% level.

There was no remarkable difference between the blood pressure falls of patients in the " benign " and those in the " malignant " phases.

There was no difference between the blood pressure falls in males and females.

In hypertensive patients, there was no apparent relationship between the extent of the blood pressure fall and the age of the patient. However, in normotensive persons there was a tendency for the large blood pressure falls to occur in older persons.

There was a tendency for the largest falls in blood pressure to occur in the subjects with highest base-line blood pressures. The few normotensive persons with large blood pressure falls had higher than average initial blood pressures. They were also above the average age of the group; so the large fall in blood pressure might have been associated equally well with either factor.

TABLE I

Effect of Intramuscular Injection of Hexamethonium Bromide (Two Milligrammes per Kilogram) in Hypertensive Subjects

Subject Number	Age (Years)	Sex	Type of Hypertension[1]	Blood Pressures (S/D)[2]						Pulse Rate		
				Base-line (Millimetres of Mercury)		After Injection (Millimetres of Mercury)		Change (Percentage of Base-line)		Base-line (Beats per Minute)	After Injection (Beats per Minute)	Change (Percentage of Base-line)
				S.[2]	D.[2]	S.	D.	S.	D.			
1	40	F.	M	240	115	160	88	−33	−23	85	79	−7
2	38	M.	M	190	119	148	102	−22	−14	70	85	+21
3	42	F.	M	190	123	148	86	−22	−30	106	86	−19
4	48	M.	B	189	104	136	92	−28	−12	62	78	+26
5	40	F.	B	196	98	131	73	−33	−25	68	78	+15
6	54	F.	M	239	138	154	96	−36	−30	95	72	−24
7	34	F.	B	222	144	153	97	−31	−33	79	102	+29
8	34	F.	M (N)	186	122	148	105	−20	−14	78	90	+15
9	40	F.	M	247	152	183	128	−26	−16	120	100	−17
10	54	F.	M	237	130	192	114	−18	−12	85	80	−6
11	44	F.	B	234	115	96	64	−59	−44	95	84	−12
12	50	F.	M	226	122	156	89	−31	−27	85	79	−7
13	47	F.	B	221	150	149	96	−32	−36	100	88	−12
14	50	M.	B	212	127	154	98	−27	−23	76	79	+4
15	35	F.	B	187	119	156	109	−17	−8	74	87	+18
16	40	F.	B	161	100	147	99	−9	−1	75	76	+1
17	27	F.	M (N)	225	129	172	114	−23	−11	95	93	−2
18	52	F.	M	231	126	191	114	−17	−10	87	78	−10
19	46	F.	M	246	132	129	86	−47	−35	107	80	−25
20	49	M.	B	201	121	172	112	−14	−7	67	86	+28
21	30	M.	B	149	133	98	89	−34	−46	109	100	−8
22	39	M.	M (N)	205	120	159	111	−23	−7	77	67	−13
23	50	F.	B	219	118	140	94	−36	−20	81	80	−1
24	59	F.	M	246	133	176	101	−28	−24	72	57	−21
25	53	M.	M	225	129	148	100	−34	−22	72	75	+4
26	43	F.	M	257	146	194	133	−25	−9	83	84	+1
27	38	F.	B	236	140	177	122	−25	−13	78	69	−12
28	39	F.	B	191	115	155	106	−19	−8	85	94	+11
29	52	F.	B	224	114	130	82	−42	−28	92	86	−7
30	37	M.	M	231	145	104	77	−55	−48	92	79	−14
31	52	M.	B	243	160	200	138	−18	−14	96	97	+1
32	39	M.	M	216	143	167	124	−23	−13	66	67	+2
33	50	F.	M	262	180	134	104	−49	−42	92	77	−16
34	46	M.	M	189	127	98	75	−48	−41	100	78	−22
35	55	F.	M	250	120	205	109	−18	−9	70	74	+6
36	40	F.	B	213	124	161	114	−24	−8	93	111	+19
37	49	M.	B	200	121	149	101	−25	−17	80	78	−2
38	63	M.	B	233	154	113	84	−51	−45	85	67	−21
39	42	M.	B	200	125	181	121	−9	−3	70	70	+0
40	37	M.	M	230	140	140	90	−39	−36	86	90	−5

[1] M, malignant; B, benign; N, nephritic.
[2] S, systolic; D, diastolic.

TABLE II

Effect of Intramuscular Injection of Hexamethonium Bromide (Two Milligrammes per Kilogram) in Normotensive Subjects

Subject Number	Age (Years)	Sex	Diagnosis	Blood Pressures						Pulse Rate		
				Base-line (Millimetres of Mercury)		After Injection (Millimetres of Mercury)		Change (Percentage of Base-line)		Base-line (Beats per Minute)	After Injection (Beats per Minute)	Change (Percentage of Base-line)
				S.[2]	D.[2]	S.	D.	S.	D.			
1	24	M.	N.C.[1]	121	72	116	74	−4	+3	77	102	+31
2	36	F.	N.C.	108	67	102	68	−6	+1	74	94	+27
3	64	M.	Arteriosclerosis	128	60	108	64	−16	+7	60	64	+7
4	72	M.	Glaucoma	146	74	75	52	−49	−30	71	69	−3
5	61	F.	Glaucoma	144	75	84	58	−42	−23	79	80	+1
6	41	M.	Acrocyanosis	115	73	114	76	−1	+4	82	88	+7
7	39	M.	N.C.	122	58	106	60	−13	+3	80	94	+17
8	34	F.	N.C.	108	59	90	61	−17	+3	72	105	+46
9	55	F.	Glaucoma	147	85	94	76	−36	−11	73	90	+23
10	39	M.	N.C.	134	80	121	85	−10	+6	94	110	+17
11	32	M.	N.C.	126	61	118	70	−6	+15	75	84	+12
12	30	F.	N.C.	107	80	92	73	−14	−9	86	115	+34
13	46	F.	N.C.	105	66	94	64	−10	−3	64	76	+19
14	43	M.	N.C.	141	93	95	71	−33	−24	89	86	−3
15	30	M.	N.C.	126	61	115	74	−9	−20	72	108	+50
16	46	F.	N.C.	112	64	96	64	−14	0	80	102	+27
17	46	F.	N.C.	132	81	90	60	−32	−26	79	84	+6
18	20	F.	Raynaud's disease	111	74	108	73	−3	+1	74	95	+15

[1] N.C., normal control.
[2] S, systolic; D, diastolic.

TABLE III

Statistical Analysis

Comparison of Hypertensive and Normotensive Subjects with Respect to Percentage Change in Blood Pressure

		Systolic		Diastolic	
		Hypertensive	Normotensive	Hypertensive	Normotensive
	Number of patients	40	18	40	18
	Mean change	−30·3	−17·5	−21·8	−3·5
Not corrected for age	Variance	166·5		185·4	
	S.E. of mean	2·04	3·04	2·15	3·19
	t	3·50		4·75	
		P<0·01		*P*<0·01	
Corrected for age	Variance	138·5		170·8	
	S.E. of mean	1·0	2·8	3·5	4·7
	t	3·5		4·7	
		P<0·01		*P*<0·01	

Comparison of Hypertensive and Normotensive Subjects with Respect to Post-Injection Blood Pressures[1]

	Systolic		Diastolic	
	Hypertensive	Normotensive	Hypertensive	Normotensive
Number of patients	40	18	40	18
Mean	152·6	101·0	100·6	68·1
Variance	755·8	167·7	288·2	68·1

[1] The variances of the blood pressures of the hypertensive and normotensive subjects are significantly different ($P<0·01$), for both systolic and diastolic pressures. Comparison of the means for the hypertensive and normotensive subjects, by means of the Aspin-Welch test, shows a significant difference ($P<0·01$) for both systolic and diastolic blood pressures.

Comparison of Hypertensive and Normotensive Subjects with Respect to Base-line Pulse Rate

	Hypertensive	Normotensive
Number of patients	40	18
Mean	85	77
Variance	143·1	
S.E. of mean	1·89	2·82
t	2·35	
	P<0·05	

Comparison of Hypertensive and Normotensive Subjects with Respect to Percentage Change in Pulse Rate

	Hypertensive	Normotensive
Number of patients	40	18
Mean	−2·0	18·5
Variance	224·6	
S.E. of mean	2·37	3·53
t	4·83	
	P<0·01	

Comparison of Hypertensive and Normotensive Subjects with Respect to Post-Injection Pulse Rate

	Hypertensive	Normotensive
Number of patients	40	18
Mean	82	91
Variance	143·5	
S.E. of mean	1·89	2·82
t	2·78	
	P<0·01	

Correlations Between Percentage Change in Blood Pressures and Percentage Change in Pulse Rate

Systolic		Diastolic	
Hypertensive	Normotensive	Hypertensive	Normotensive
r=0·502	0·490	0·505	0·597
P<0·01	*P*<0·05	*P*<0·01	*P*<0·05
(38 *d.f.*)	(16 *d.f.*)	(38 *d.f.*)	(16 *d.f.*)

The mean post-injection blood pressure levels, both systolic and diastolic, were considerably higher for the hypertensive than for the normotensive subjects. The post-injection blood pressure levels of the hypertensive persons were more variable than those of the normotensive persons. The differences between the means and the differences between the variances were all statistically significant at the 1% level.

Pulse Rate

The base-line pulse rate was slightly higher in the hypertensive than in the normotensive subjects. The difference was significant only at the 5% level.

After the injection of methonium there was little change in pulse rate in the hypertensive subjects; in the normotensive subjects there was a rise in pulse rate (Figure II). The

difference between the means of the changes is statistically significant at the 1% level.

The mean post-injection pulse rate was higher in the normotensive than in the hypertensive subjects. The difference between the means is significant at the 1% level.

the percentage change in both the systolic and diastolic blood pressure and the percentage change in pulse rate are significant at the 1% level. In the normotensive subjects, these correlations are significant only at the 5% level.)

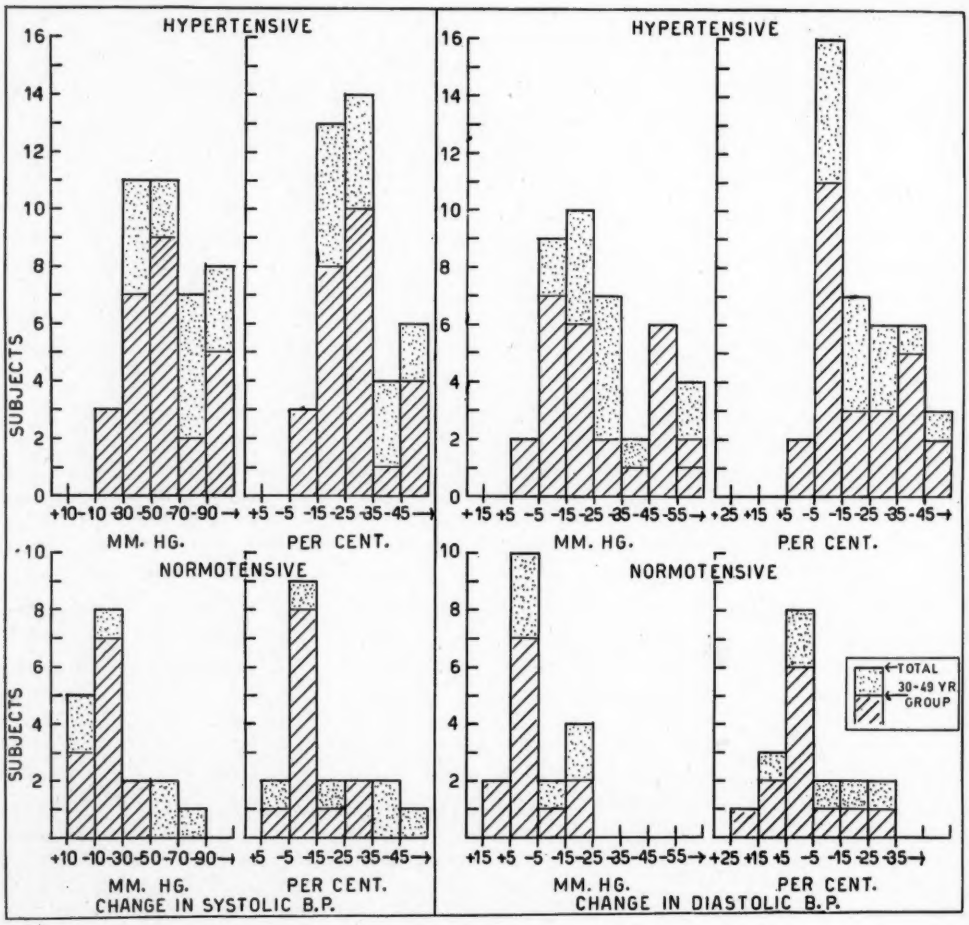

FIGURE I

Blood pressure changes, in millimetres of mercury and as percentages of base-line values, resulting from the injection of hexamethonium bromide in normotensive and hypertensive subjects

Correlation between Change in Blood Pressures and Change in Pulse Rate

In both the hypertensive and normotensive subjects, there was a tendency for those with the greatest rise in pulse rate to have the least fall in blood pressure (Figure III). (In the hypertensive subjects, the correlations between

DISCUSSION

Since the above observations were made, similar findings have been reported by Conway (1953), who gave intravenous injections of hexamethonium bromide to 46 hypertensive and 12 normotensive persons. The fall in the diastolic blood pressure was greater in the

hypertensive subjects; the most marked falls occurred in older patients. He considered that his results showed that there was a neurogenic component in hypertension in the elderly.

Our observations indicate that the responses of hypertensive and of normotensive persons to the intramuscular injection of hexamethonium bromide differ in two respects: firstly, there is a greater proportional fall in both the systolic and diastolic blood pressure in hypertensive persons; secondly, whereas in hypertensive persons there is usually no change or a slight fall in pulse rate, in normotensive persons the pulse rate usually rises.

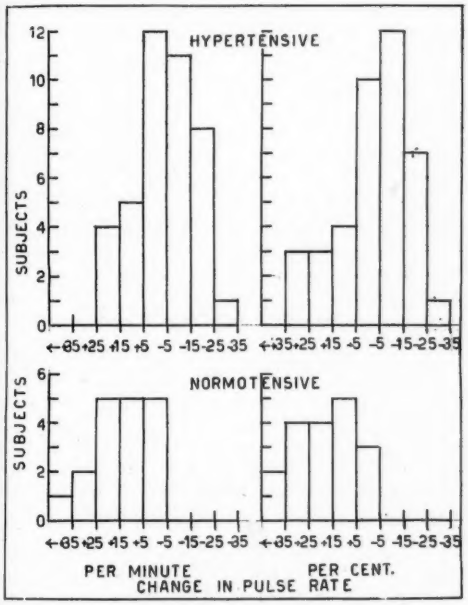

FIGURE II

Pulse rate changes resulting from the injection of hexamethonium bromide, both as beats per minute and as percentage of base-line values, in normotensive and hypertensive subjects

It may be asked whether there is any causal relationship between the difference in the responses in the blood pressure in the two groups on the one hand and the difference in the responses of the pulse rate on the other.

A fall in blood pressure in a subject with nervous pathways intact would be expected to produce an increase in pulse rate—from release of vagal tone and, in certain instances, from increased cardiac sympathetic nervous activity. However, in a subject who has received methonium both the sympathetic and parasympathetic pathways are "blocked", and the pulse rate would be expected to have the rate of a denervated heart and to be not greatly affected by the level of the blood pressure. In the subjects with the greatest fall in blood pressure there was the least rise (or greatest fall)

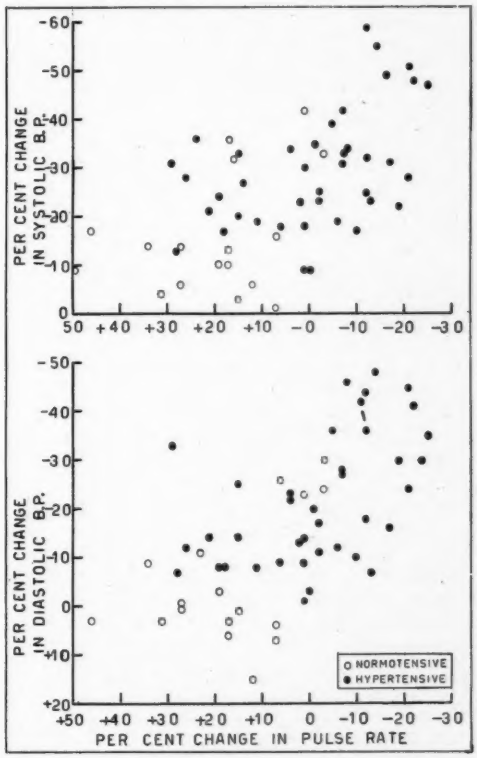

FIGURE III

Relationship of percentage change in blood pressure to percentage change in pulse rate following the injection of hexamethonium bromide

in pulse rate. For these two reasons, it is most unlikely that the different response of the blood pressure in the two groups was responsible for the different response of the pulse rate.

A second possibility is that the difference in response of the pulse rate in the two groups is responsible for the difference in response in the blood pressure. If the output of the heart remains unchanged (see later), an increased pulse rate would be expected to produce a narrowing of the pulse pressure with no change in the mean blood pressure; the systolic blood

pressure would be expected to fall and the diastolic blood pressure to rise. Thus the increased pulse rate might be partly responsible for the relatively small fall in the diastolic blood pressure in the normotensive compared with that in the hypertensive persons. It would not account for the relatively small fall in the systolic blood pressure. In fact, the trend of the relationship between the pulse rate change and the blood pressure change was the same in respect to both the systolic and the diastolic blood pressures; the higher the rise in pulse rate, the less the fall in blood pressure.

There is a third possibility—which by exclusion is the most likely—that both the difference in response of the blood pressures and the difference in response of the pulse rate in the two groups were related to some other factor. This will be discussed shortly.

At this point, the discussion is simplified by considering the effects on the blood pressure and pulse rate separately. The fall in blood pressure from the injection of methonium might result from a decreased peripheral resistance or a decreased cardiac output. Gilmore and his co-workers (1952), in observations in 14 hypertensive subjects and one normotensive person, found that the cardiac output determined by cardiac catheterization was not significantly altered after the injection of methonium. The fall in blood pressure must be due to a fall in peripheral resistance. The calibre of the peripheral vessels, which determines the peripheral resistance, is under the control of the sympathetic nervous system. It therefore appears that sympathetic nervous blockade produces a greater fall in the peripheral resistance in hypertensive than in normotensive persons. This might be interpreted as evidence for increased sympathetic nervous activity in the hypertensive subjects. The increased sympathetic effect could just as readily be explained by increased responsiveness to a normal degree of sympathetic activity. It is not possible on the available evidence to decide between these possibilities. If there were increased sympathetic nervous activity, it would be expected that there would be increased production of the sympathetic nervous transmitter — noradrenaline. However, the available evidence indicates that the noradrenaline excretion in hypertensive persons is not raised (von Euler, 1951; Burn, 1953). There is some evidence that the blood vessels of hypertensive persons react excessively to normal stimuli. Thus, Goldenberg and his co-workers (1948) found that the intravenous infusion of noradrenaline produced a greater pressor effect in hypertensive than in normotensive persons.

The different effect on the pulse rates is not likely to be due to removal of sympathetic tone for the following reasons: (*a*) in the resting subject the pulse rate is mainly controlled by vagal activity, so that there is little sympathetic tone to release; (*b*) the commonest finding in the normotensive subjects is an increase in pulse rate, which could not occur from removal of sympathetic influence. It is therefore probable that the changes in pulse rate are mainly due to removal of vagal restraint. Since a greater rise in pulse rate occurs in normotensive than in hypertensive subjects, it appears that vagal activity is greater in normotensive persons. Some support for this suggestion is given by the observation that the base-line pulse rate in the hypertensive subjects was slightly greater than in the normotensive persons (instead of less as might have been expected with a normally functioning carotid sinus mechanism). A decreased responsiveness of the carotid sinus could result in both decreased vagal effect on the pulse rate and increased sympathetic action (from lack of the depressor reflex). Heymans and van den Heuvel-Heymans (1951), from a study of the effect of tone of the carotid region on the reflexes controlling blood pressure, have suggested that decrease of tone and resistance to stretch of this region could be the mechanism of arterial hypertension.

We do not suggest that the increased sympathetic effect is the sole mechanism of arterial hypertension. If this was so, blockade of the sympathetic nerves would cause the blood pressure to return to the same level in hypertensive as in normotensive persons. This does not occur.

We cannot say at present whether the increased sympathetic effect is a primary or secondary mechanism. If excessive sympathetic effect caused hypertension, this could produce in turn secondary changes in the vessels and in the kidneys which could perpetuate the hypertension. Conversely, if hypertension resulted from some other cause, this might conceivably produce degenerative changes in the carotid sinus, which would in turn lessen the depressor effect on the vasomotor centre.

SUMMARY AND CONCLUSIONS

From a study of physical principles and reported observations, we reach the conclusion that the probable cause of arterial hypertension is increased peripheral resistance due to a functional narrowing of the arterioles, together with a normal volumetric cardiac output.

We have described the effect of the intramuscular injection of hexamethonium bromide (two milligrammes per kilogram) in 40 hypertensive and 18 normotensive persons. This had the following results: (*a*) a greater relative fall in both systolic and diastolic blood pressures in the hypertensive subjects; (*b*) commonly, a rise in pulse rate in the normotensive subjects, but usually no change or a slight fall in pulse rate in the hypertensive subjects.

The probable significance of these findings is as follows: (*a*) The blood pressure changes indicate an increased sympathetic nervous effect in hypertensive compared with normotensive subjects. This does not necessarily mean increased sympathetic nervous activity; it could occur just as readily from excessive responsiveness of the arterioles to normal sympathetic nervous activity. (*b*) The pulse rate changes indicate a decreased vagal effect in hypertensive subjects. (*c*) Both excessive sympathetic nervous activity and reduced vagal activity might arise from decreased responsiveness of the carotid sinus mechanism.

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